

Evaluation of *Moringa oleifera*(Lam.) and *Moringa peregrina*(Forssk.)leaf extracts for their antioxidant activity, anti-breast cancer potential, and diversity in mineral concentrations

Fouad Ibrahim¹, Awad F. Elsheikh², Mahmoud A Al-Saman³, Ibrahim E Mousa⁴, Rafaat M Elsanhoty^{1*}, Gomaa Sulaiman⁵, Idress Hamad Attitalla⁶, Waeil I.T Kawafi⁷

¹Department of Biochemistry, Faculty of Medicine Almaraj, Benghazi University, Libya.

²Department of chemistry, school of basic science, Libyan Academy - Benghazi

³Department of Industrial Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Sadat City 22857, Egypt

⁴Department of Environmental Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Sadat City 22857, Egypt

⁵Faculty of Medical Technology, University of Tripoli (UOT), Tripoli, Libya.

⁶Department of Microbiology, Faculty of Medical Technology (Dean), Box 919, Al-Bayda, Libya.

⁷Department of Community medicine, Faculty of Medicine Almaraj, Benghazi University, Libya

ABSTRACT

Moringa oleifera (Lam., L.) and *Moringa peregrina* (Forssk., F.) are perennial angiosperm plants that are members of the Moringaceae family. The goal of this work was to explore the bioactive properties of *M.oleifera* and *M. peregrina* leaves. Antioxidant activity (DPPH) and mineral composition (ICP-MS) were determined. Also, to investigate whether different extracts of *M.oleifera* and *M. peregrina* leaves have an effect on human breast cancer cell line MDA-MB-231. The highest antioxidant activities with *M. oleifera* and *M. peregrina* extracts were obtained using ethanol and methanol extracts, while the lowest activity was observed with *n*-hexane. The antioxidant activity increases with increasing concentration of extract. The IC₅₀ value of *M. oleifera* ethyl acetate extract was 58.25 µg/mL, followed by the ethanol extract 98.21 µg/mL. The IC₅₀ value of *M. peregrina* methanol extract was 97.14 µg/mL, followed by the ethanol extract 214.38 µg/mL. *M. oleifera* and *M. peregrina* leaves were found to be a good supplement of K, Ca and Fe. Ethyl acetate crude extract of *M. oleifera* exhibited a positive effect on the MDA-MB-468 cell line with IC₅₀ value of 8.49. Moreover, methanol crude extract of *M. peregrina* presented a positive effect on the MDA-MB-468 cell line with IC₅₀ values of 7.74. Staurosporine “positive control” showed cytotoxicity to breast cell line with IC₅₀ values of 5.001 µg/mL. These results indicate that the *M. peregrina* methanol extract has anti-cancer activity that can be used to improve novel drugs for breast cancer treatment.

Keywords: *Moringaoleifera*, *Moringaperegrina*, antioxidant, breast cancer, cytotoxicity, ICP



*Corresponding Author

Rafaat M Elsanhoty

Department of Biochemistry, Faculty of Medicine Almaraj, Benghazi University, Libya.

© Copy Right, IJMPS, 2024. All Rights Reserved

1. INTRODUCTION

Moringa oleifera(Lam., L.) and *Moringa peregrina* (Forssk., F.) are two names for the perennial angiosperm plant that belong to the family Moringaceae (Olson, 2002; Ferreira et al. 2014), which includes many diverse species. It is extensively grown in all tropical and subtropical countries around the world (Mbikay, 2012). Throughout history, the plant has been utilized as a traditional medicine to treat a wide range of ailments, including skin conditions, respiratory disorders, ear, and tooth infections, hypertension, diabetes, anemia, and cancer (Jung, 2014; Tiloke et al. 2013). Leone et al. (2015) provided a thorough description of the pharmacological significance of the leaf extract that contains bioactive chemicals. The deciduous *M. peregrina* tree may reach 3-10 m in height and has grey-green bark. This plant has been examined for a number of pharmacological properties and has a broad variety of traditional medicinal applications (Saleh et al. 2017; Azim et al. 2017). *M. peregrina* is utilized in folk medicine due to its therapeutic properties for treating fever, headache, constipation, back and muscular pain, burns and slimness. It is also used to treat skin rashes, wound healing, diabetes and disinfection (Al-Khatani, 1995; Elbatran et al.2005; Nawash and Ahmad, 2011).

One of the major causes of death for humans is cancer. The high rate of cancer cases is caused by a number of factors, including poor diet, pollution, inactivity, and tobacco product usage (WHO, 2018). Various therapeutic approaches, including radiotherapy, chemotherapy, oncological surgery, immunotherapy, and stem cell transformation, have been used depending on the location and stage of the tumors. Chemotherapy is a kind of treatment that is not very effective and also affects healthy body cells that proliferate fast under normal circumstances, such as bone marrow, hair follicle cells, and the digestive tract. The negative effects support the need for a back-to-nature strategy in cancer treatment (Singh et al. 2016; Adnan et al. 2021).

Worldwide, breast cancer in women is one of the deadliest types of cancer (ACS, 2013). The ability of cancer cells to multiply, penetrate through extracellular matrix, and move to different areas of the body to create new tumors are some of their main distinguishing characteristics. The tumor microenvironment provides nutrients and support to malignant cells, enabling them to migrate and create new blood vessels through a process known as angiogenesis (Folkman, 1982). Designing the optimal treatment plan that kills the greatest number of cancer cells with the least amount of adverse effects and maximizes benefits for cancer patients is a challenging problem for oncologists and medical scientists. Most of the recognized anticancer drugs are derived from different plant species (Craig and Beck, 1999). There are several nutritional products that show anticancer potential with minimal side effect and are currently undergoing clinical trials for cancer treatment (Garg et al. 2005); for example curcumin and lycopene. Curcumin is a polyphenolic compound isolated from turmeric and exhibits antimicrobial, immunomodulatory, and anticancer chemopreventive efficacy (Sa et al. 2010). Lycopene is a carotenoid compound abundant in tomatoes (Kong et al. 2010).

Various scattered studies have been reported on extracts using different parts of different Moringa species, but different polar and non-polar solvents such as chloroform, acetone, methanol and hexane on the leaves are novel in the present study. In this study we focused upon the effect of *M. oleifera* and *M. peregrina* extracts from leaves to observe its efficacy as an anti-cancer agent on breast cancer. Therefore, the aim of this study was to evaluate the antioxidant activity and anti-cancer potential against breast cancer cell line of two species of Moringa (*M. oleifera* and *M. peregrina*) grown in Egypt. The importance of this study lies in the fact that this locally grown plant has not been previously tested as an anti-cancer agent.

2. MATERIALS AND METHODS

Plant collection and preparation

Moringa species (*M. oleifera* and *M. peregrina*) used in this study were obtained from Moringa Production Unit (MPU), National Research Centre (NRC), Giza, Egypt, in September 2022. Fresh leaves of the plant were separated, cleaned with distilled water, and permitted to air dry in the shadow. The dry matter was pulverized to a fine powder (60 mesh size) and stored at 4°C in brown glass bottles until use in subsequent tests.

Maceration process

Ten g of samples leaf powder were extracted by shaking at 150 rpm for 24 h at room temperature (RT) with 100 mL of solvent (methanol, 70% ethanol, acetone, chloroform, ethyl acetate and *n*-hexane), based on the technique described by Romani et al. (2006). The extracts were filtered in a Buchner funnel using Whatman No. 1 filter paper. The residue was extracted again with 50 mL of solvent and then filtered, and the filtrates were collected and evaporated under reduced pressure in a rotary evaporator (Heidolph VV 2000). It was then vacuum-dried in a desiccator to obtain a constant weight (Laaksonen et al. 2002). The weights of the final extracts were calculated. The residue was finally re-dissolved in a minimum volume of DEMSO solvent to produce a concentration of 10 mg/mL. The extracts were stored at 4°C prior to use.

DPPH radical method

Free radical scavenging activity was determined by DPPH assay. The reaction combination contained 100 µL of test extracts (1000 to 62.5 µg) and 1 mL of a methanol solution of 0.2 mM DPPH radical. After shaking, the mixture was incubated for 30 min at 37°C. Ascorbic acid was used as a positive control; Absorbance was measured at 517 nm. According to the equation below, the reaction mixture with lower absorbance has higher free radical scavenging activity.

DPPH scavenging activity (%) = 100 x (A₀ - A₁) / (A₀)

Where: A₀ is the absorbance of the control, A₁ is the absorbance of mixture containing DPPH and extract at 517 nm (Al-Saman et al. 2019).

Mineral content determination

Using ICP-MS (iCAP, Thermo, Germany); the mineral composition of the leaves was identified. Approved reference materials (Merck, Germany) were used. The mean and relative standard deviations were determined using Qtegra software (Eaton and Franson, 2005).

Cell culture condition

The MDA-MB-231 breast cancer cell line was purchased from ATCC (Manassas, VA, USA). The cells were grown in DMEM (Dulbecco's Modified Eagle's Medium; Invitrogen) supplemented with 10% FBS (fetal bovine serum; Hyclone), 50 U/mL penicillin, and 50 µg/mL streptomycin. All cultures were incubated at 37°C in an atmosphere containing 5% CO₂ and 95% air.

Cell viability (MTT assay)

The MTT assay was used to determine whether moringa leaf extracts inhibit cancer cell proliferation, as previously reported (Lee and Houghton, 2005). MDA-MB-231 cells were plated onto 96-well plates and left to attach for 24 hrs. The moringa leaf extracts were dissolved in 0.1% DMSO and serially diluted with medium. Cells were treated with moringa leaf extracts at various doses and incubated for 72 h. The crude extract is typically thought to exhibit *in vitro* cytotoxic effect with an IC₅₀ value 20 µg/mL, according to the plant screening program of the United States National Cancer Institute (US-NCI) (Sriwiriyan et al. 2014).

Statistical analysis

An analysis of variance (ANOVA) was performed on all data. Three samples of each item were examined, and main values and the SD were provided. Duncan's multiple range tests were used to examine the significance of the variable mean differences (p ≤ 0.05). SPSS 16 software was used for all analyses.

3. RESULTS AND DISCUSSION

Effect of extraction solvent on phytochemical content

The results showed a significant difference in extraction yield when a range of solvents were used. The maximum extraction yield with *M. oleifera* was 22.0% for ethanol, followed by 15.0% for methanol, 6.0 and 40% for chloroform and *n*-hexane, respectively; on the other hand the maximum extraction yield with *M. peregrina* was 21.0% for ethanol, followed by 14.0% for methanol, 3.0% for chloroform and *n*-hexane, results indicating that highly polar solvents had the best extraction efficiency.

Antioxidant activity

To learn more about the potential health benefits of the Moringa species under study, we looked at the antioxidant capabilities of several Moringaleaf extracts. **Table 1A** shows that DPPH radical was scavenged by all *M. oleifera* extracts. The highest scavenging power was demonstrated with ethanol extract (87.78%), followed by methanol (85.68%), then acetone (79.50%). On the other hand, *n*-hexane showed the lowest scavenging efficiency (47.15%) of all extraction solvents. It was observed that the scavenging effectiveness increased with increasing concentration of *M. oleifera*. According to statistical analysis, a concentration of 1000 µg/µL led to the highest scavenging (92.05%), followed by 500 µg/µL (78.05%) and 250 µg/100 µL (72.92%), respectively. The lowest scavenging activity was found at a concentration of 65 µg/100 µL with an average value of 53.50%. According to **Table 2**, the IC₅₀ values for the methanol extract were 107.5 µg/mL, the ethanol extract was 98.21 µg/mL, and the acetone extract was 2767.66 µg/mL. Statistical analysis showed no significant changes between methanol and 70% ethanol extracts.

Table 1B shows that DPPH radical was scavenged by all *M. peregrina* extracts. The highest scavenging power was shown with ethanol extract (96.44%), followed by methanol (95.35%), then acetone (88.28%). On the other hand, *n*-hexane showed the lowest scavenging efficiency (73.24%) of all extraction solvents. It was observed that the scavenging force increased with increasing concentration of *M. peregrina*. According to statistical analysis, a concentration of 1000 µg/µL led to the highest scavenging (92.86%), followed by 500 µg/µL (87.66%) and 250 µg/100 µL (84.70%), respectively. The lowest scavenging activity was found at a concentration of 65 µg/100 µL with an average value of 78.40%. Based on **Table 2**, the IC₅₀ values for the methanol extract were 97.14 µg/mL, the ethanol extract was 214.38 µg/mL, and the acetone extract was 166.30 µg/mL. Statistical analysis showed no significant changes between methanol, 70% ethanol and acetone extracts.

Growing concerns about the harmful effects of synthetic medications and antioxidants on health have led to the identification of naturally occurring bioactive principles found in herbs and plants, which are thought to have significant activity against harmful reactive oxygen species. One of the most popular and extensively utilized plants for antioxidant purposes is *M. oleifera*. Nevertheless, no research has demonstrated the simultaneous assessment and comparison of unique extracts of different solvents from different plant parts. All extracts in this research had a notable level of activity, with the 70% ethanolic extract of *M. oleifera* and *M. peregrina* leaves showed the greatest antioxidant activity (P < 0.05). There is a strong correlation between an extract's polyphenolic concentration and its antioxidant activity. The results of this

investigation are supported by the fact that these bioactive compounds may be maximally extracted from solvents with intermediate polarity, including ethanol and methanol (Chenielle et al. 2009).

The results of our work are consistent with the observation that polyphenolic bioactive constituents may be optimally extracted by 80% and 70% methanolic and ethanolic solvents, respectively (Siddhuraju and Becker, 2003). Furthermore, phenolic compounds in plant extracts may have served as effective radical scavenging and reducing agents, according to Qader et al. (2011). All antioxidant test results in this study point to ethanolic extracts as potential effective replacements for synthetic antioxidants, which may have detrimental effects on both human and animal health. Methanolic extraction may leave residues that cause toxicity even at low doses, which makes the ethanolic extract more appropriate for nutraceutical uses (Paine and Davan, 2001). The flavonoid rutin that was extracted from this plant and the phenolic content of the *M. peregrina* leaf extract (Iqbal and Bhangar, 2006) may be responsible for the antioxidant activity that has been demonstrated in vitro (Siddhuraju and Becker, 2003).

Minerals content in leaves of *Moringa* species

Figure 1A shows the average mineral composition concentration and range of values for all *Moringa* samples using chemical reference methods (ICP-MS). Macrominerals such as K, Ca and Mg were found in high amounts in *M. oleifera* leaves (818.682 mg/kg “22%”, 445.915 mg/kg “12%” and 233.045 mg/kg “6%”, respectively based on a dry weight). Other microminerals such as Fe, Co, Cu, Mn and Zn were found in trace amounts (539.101 “15%”, 2.350, 12.582, 7.464 and 27.570 mg/kg DW, respectively). Figure 1B shows the average mineral composition concentration and range of values in *M. peregrina* leaves. Macrominerals such as Ca, K and Mg were found in high quantities in *M. peregrina* leaves (722.058 mg/kg “21%”, 549.561 mg/kg “16%” and 403.841 mg/kg “12%”, respectively). Other microminerals such as Fe, Cu, Mn and Zn were found in trace amounts (329.163 “10%”, 20.502, 7.712 and 53.659 mg/kg DW, respectively).

This study is the first comprehensive analysis of element concentrations in leaves of *M. oleifera* and *M. peregrina*. Variation in *M. oleifera* and *M. peregrina* leaf element concentrations can be the result of environment/management influence, the influence of genetic variation within and between species (Olson et al. 2016), and interactions between genetics and environment (Melesse et al. 2012). Climate and soil type may also contribute to variation in the concentration of elements in leaves. The positive correlation between the concentration of elements in *Moringa* species and the chemical properties of the soil indicate the significance of the soil environment in which the plants grow, in addition to the inherent genetic ability of these species to absorb mineral elements and transfer them to edible parts. Previous studies reported that *Moringa* leaves have K content more than bananas by 15 times, Ca content more than milk by 17 times, and Fe content more than spinach by 25 times (Said-al et al. 2017; Osman and Abohassan, 2012; Rockwood et al. 2013). Our findings indicate that *Moringa* leaves are safe to eat and that levels were within the permissible range for human consumption.

Cytotoxic effect of crude extracts of *Moringa* species on breast cancer cell line

Figures 2 (A and B) show the results of IC₅₀ values of different *Moringa* extracts against human breast cancer cell line MDA-MB-231. Methanol, 70% ethanol, acetone, chloroform, ethyl acetate and *n*-hexane solvents were used to extract active constituents from the *M. oleifera* leaves, and MTT was used to determine their cytotoxic effects against a breast cancer cell line (MDA-MB-231). After treatment, the ethyl acetate, methanol, acetone and *n*-hexane crude extracts represented positive effects on the MDA-MB-468 cell line with IC₅₀ values of 8.49, 24.17, 30.44 and 35.81 µg/mL, respectively (Figure 2A); while crude extracts of ethanol and chloroform confirmed a weak effect on the cell line. Staurosporine “positive control” showed cytotoxicity to breast cell line with IC₅₀ values of 5.001 µg/mL. The results indicate that the best solvent for the extraction of the active compounds was ethyl acetate.

After treatment with *M. peregrina* extracts, the crude extracts of methanol, acetone, and *n*-hexane presented positive effects on the MDA-MB-468 cell line with IC₅₀ values of 7.74, 11.19 and 14.57 µg/mL, respectively (Figure 2b); while crude extracts of ethyl acetate, chloroform and ethanol confirmed a weak effect on the cell line. Staurosporine “positive control” showed cytotoxicity to breast cell line with IC₅₀ values of 5.001 µg/mL. The results indicate that the best solvent for extracting the active compounds was methanol.

In this study, we evaluated the anti-cancer properties of *M. oleifera* and *M. peregrina* leaves extracts against MDA-MB-231 breast cancer cell line. *M. oleifera*, a common vegetable plant in many countries e.g. Asian and South East Asian countries; possesses numerous compounds with excellent health benefits including anti-oxidant and anti-cancer properties (Abdull Razis et al. 2014). The plant exhibits anti-cancer potential by interfering with the signal transduction cascade that promotes cancer cell proliferation and progression (Tiloke et al. 2013). The inhibition of cancer cell proliferation is mainly due to the presence of eugenol, a phenolic natural compound (Al-Sharif et al. 2013), D-allose (Sui et al. 2005), isopropyl

isothiocyanate (Matsuda et al. 2007). Keeping in view the anti-cancer properties of *M. oleifera* we hypothesized it may be an effective treatment for breast cancer. There is evidence that D-allose (present in leaves of Moringa) inhibits the growth of cancer cells at G1 phase (G1- cell cycle arrest) without exerting appreciable effects on normal cells (Yamaguchi et al. 2008). The presence of isothiocyanate (organic sulphur compound) in *M. oleifera* bark extract can be attributed to its anti-cancer property.

Hexadecanoic acid (palmitic acid) present in all parts (leaf, seed and bark) of Moringa has been found to have selective cytotoxicity against human leukemic cells, as well as *in vivo* anti-tumor activity in mice (Harada et al. 2001). The extract of Moringa leaves have a number of bioactive anti-cancer constituents which might be responsible for its strong anti-cancer activity against MDA-MB-231 cancer cell line. Our results are in good agreement with previous work demonstrating *M. oleifera* leaves possessed anti-cancer properties (Jung, 2014). Al-Sharif et al. (2013) reported that the possible mechanism of action of eugenol by down regulating the E2F1 protein which shows promising outcomes in breast cancer.

Al Kaabi et al. (2022) reported that *M. peregrina* possessed strong anti-cancerous activity against breast and colon cancer cell lines, which might be due to the presence of promising anti-cancer compounds in the tuber, leaves and stem of the plant. Thus, it is clear that non-polar solvent chloroform is able to dissolve the active compounds from the source to the extract of *M. peregrina*, and it showed maximum anti-cancer properties compared to other extracts.

The implication of this study opens a new and fertile area of future research to elucidate the molecular mechanism by which signaling events take place after treating breast cancer cells with *M. oleifera* and *M. peregrina* extracts. Also, *in vivo* pharmacological and toxicological studies are required to prove the unexplored beneficial aspects of these medicinal plants.

CONCLUSION

Antioxidants are essential to the human body's ability to survive. The ability of six leaf extracts from *M. oleifera* and *M. peregrina* to scavenge free radicals was tested. Methanolic and ethanolic extracts of *M. oleifera* and *M. peregrina* had significant activity in DPPH. The leaves of *M. oleifera* and *M. peregrina* possessed potent antioxidant activity and can be used as a source of natural antioxidants. *M. oleifera* and *M. peregrina* leaves were found to be a good supplement of K, Ca and Fe. Based on the current research, it can be concluded that *M. peregrina* (followed by *M. oleifera*) showed high anticancer activity against breast cancer cells. This may be because the plant's leaves contain active constituents that have the potential to fight cancer. It is evident from this that methanol, a polar solvent, can effectively extract active compounds and has the most anti-cancer effects when compared to other extracts. It will take *in vivo* pharmacological and toxicological investigations to prove the undiscovered health benefits of this therapeutic plant. The extraction and identification of anticancer compounds from *M. peregrina* plant leaves needs further investigation.

Author Contributions:

All authors listed have contributed significantly to the development and the writing of this article.

Ethics Approval and Consent To Participate:

All applicable international, national, and/or institutional guidelines for the use of cell lines were followed.

Consent For Publication:

All authors consent for the manuscript to be published.

Funding:

The research received no external funding.

Conflicts Of Interest:

The authors declare no conflict of interest.

REFERENCES

1. Olson ME. Combining data from DNA sequences and morphology for a phylogeny of Moringaceae (Brassicales). *Systematic Botany*. 2002; 27(1):55-73
2. Paulo Michel Pinheiro Ferreira, Éverton José Ferreira de Araújo, Jurandy do Nascimento Silva, Rivelilson Mendes de Freitas, Nagilla Daniela de Jesus Costa, Samara Ferreira de Carvalho Oliveira, et al. Safety and Efficacy of *Moringa oleifera* Lamarck (1785)-Therapeutic and Toxicological Properties. In: Gowder DSJT, editor. *Pharmacology and Therapeutics*, InTech; 2014

3. Mbikay M. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: a review. *Frontiers in pharmacology*. 2012; 3
4. Jung IL. Soluble extract from *Moringa oleifera* leaves with a new anticancer activity. *PloS one*. 2014; 9(4):e95492. doi: 10.1371/journal.pone.0095492 PMID: 24748376; PubMed Central PMCID: PMC3991666
5. Tiloke C, Phulukdaree A, Chuturgoon AA. The antiproliferative effect of *Moringa oleifera* crude aqueous leaf extract on cancerous human alveolar epithelial cells. *BMC complementary and alternative medicine*. 2013; 13(1):226
6. Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. Cultivation, Genetic, Ethnopharmacology, Phytochemistry and Pharmacology of *Moringa oleifera* Leaves: An Overview. *International journal of molecular sciences*. 2015; 16(6):12791–835. doi: 10.3390/ijms160612791 PMID: 26057747
7. Saleh NM, Mabrouk MI, Salem-Bekhit MM, Hafez EH. Challenge of *Moringa peregrina* Forssk as an antimicrobial agent against multi-drug-resistant *Salmonella* sp. *Biotechnol. Equip*. **2017**, 31, 380–386
8. Azim SAA, Abdelrahem MT, Said MM, Khattab A. Protective effect of *Moringa peregrina* leaves extract on acetaminophen-induced liver toxicity in albino rats. *Afr. J. Tradit. Complement. Altern. Med*. **2017**, 14, 206-216
9. Nawash OS, Ahmad Al-S H. The most important medicinal plants in Wadi Araba desert in South West Jordan: A review article. *Adv. Env. Biol*. **2011**, 418-426
10. Al-Khatani H. *Moringa peregrina* (Al-yassar of Al-ban) seeds oil from northwest Saudi Arabia. *J. King Saud Univ. Agri. Sci*. **1995**, 7, 31-45
11. Elbatran SA, Abdel-Salam OM, Abdelshfeek KA, Nazif NM, Ismail SI, Hammouda FM. Phytochemical and pharmacological investigations on *Moringa peregrina* (Forssk) Fiori. *Nat. Prod. Sci*. **2005**, 11, 199-206
12. WHO. Global cancer data. In *International Agency for Research on Cancer World Health Organization*; WHO: Geneva, Switzerland, 2018; 263
13. Singh S, Sharma B, Kanwar SS, Kumar A. Lead phytochemicals for anticancer drug development. *Front. Plant Sci*. 2016, 7, 1667
14. Adnan M, Siddiqui AJ, Hamadou WS, Snoussi M, Badraoui R, Ashraf SA, Jamal, A, Awadelkareem AM, Sachidanandan M, Hadi S. et al. Deciphering the Molecular Mechanism Responsible for Efficiently Inhibiting Metastasis of Human Non-Small Cell Lung and Colorectal Cancer Cells Targeting the Matrix Metalloproteinases by *Selaginella repanda*. *Plants* 2021, 10,979
15. ACS. Breast Cancer Facts and Figures. American Cancer Society. 2013, 14:1-40
16. Folkman J. Angiogenesis: initiation and control. *Annals of the New York Academy of Sciences*. 1982; 401:212–27. PMID: 6188401
17. Craig W, Beck L. Phytochemicals: Health Protective Effects. *Canadian journal of dietetic practice and research: a publication of Dietitians of Canada Revue canadienne de la pratique et de la recherché en dietetique: une publication des Dietetistes du Canada*. 1999; 60(2):78-84. PMID: 11551345
18. Garg AK, Buchholz TA, Aggarwal BB. Chemosensitization and radiosensitization of tumors by plant polyphenols. *Antioxidants & redox signaling*. 2005; 7(11-12):1630-47
19. Sa G, Das T, Banerjee S, Chakraborty J. Curcumin: from exotic spice to modern anticancer drug. *Al Ameen J Med Sci*. 2010; 3:21-37
20. Kong K-W, Khoo H-E, Prasad KN, Ismail A, Tan C-P, Rajab NF. Revealing the power of the natural red pigment lycopene. *Molecules*. 2010; 15(2):959-87 doi: 10.3390/molecules15020959 PMID: 20335956
21. Al-Saman MA, Abdella A, Mazrou KE, Tayel AA, Irmak S. 2019. Antimicrobial and antioxidant activities of different extracts of the peel of kumquat (*Citrusjaponica* Thunb). *J. Food Meas. Charact*. 13, 3221-3229
22. Eaton AD, Franson MAH, American Public Health Association (APHA), *Standar Methods for the Examination of Water & Wastewater* (Am. Public Heal. Assoc. Am. Water Work. Assoc. Water Environ. Fed, Washington, 2005)
23. Laaksonen TJ, Roos VH, Labuza TP. 2002. Comparisons of the use of desiccators with or without vacuum for water sorption and glass transition studies. *Int. J. Food Prop.*, 4, 545-563
24. Lee CC, Houghton P (2005). Cytotoxicity of plants from Malaysia and Thailand used traditionally to treat cancer. *J Ethnopharmacol* 100:237–243.
25. Romani A, Pinelli P, Cantini C, Cimato A, Heimler D. 2006. Characterization of Violetto di Toscana, a typical Italian variety of artichoke (*Cynarascolymus* L.). *Food Chem*. 95, 221-225
26. Sriwiryajan S, Ninpesh T, Sukpondma Y, Nasomyon T, Graidist P. 2014. Cytotoxicity screening of plants of genus *Piper* in breast cancer cell lines. *Trop. J. Pharm. Res*. 13, 921-928
27. Chenielle D, Lois R, Alison N, Sylvia M, John L, Mohammed A. (2009). Antibacterial and antifungal analysis of crude extracts from the leaves of *Callistemon viminalis*. *Journal of Medical and Biological Science*, 3(1), 1-7
28. Qader S Wasman, Abdulla MA, Lee SC, Nigar N, Zain MM, Hamdan S (2011). Antioxidant, Total Phenolic Content and Cytotoxicity Evaluation of Selected Malaysian Plants. *Molecules*, 16, 3433-3443

29. Paine A, Davan AD (2001). Defining a tolerable concentration of methanol in alcoholic drinks. *Hum Exp Toxicol*, (11), 563-8.
30. Iqbal S, Bhanger M (2006). Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *J Food Comp Anal*. 19:544-551
31. Siddhuraju P, Becker K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J Agr Food Chem*. 51:2144-2155.
32. Olson ME, Sankaran RP, Fahey JW, Grusak MA, Odee D, Nouman W. Leaf protein and mineral concentrations across the "miracle tree" genus *Moringa*. *PLOS ONE*. 2016; 11(7):e0159782. <https://doi.org/10.1371/journal.pone.0159782> PMID: 27459315
33. Melesse A, Steingass H, Boguhn J, Schollenberger M, Rodehutschord M. Effects of elevation and season on nutrient composition of leaves and green pods of *Moringa stenopetala* and *Moringa oleifera*. *Agrofor Syst*. 2012; 86(3):505±18.
34. Said-al Ahl HAH, Hikal WM, Mahmoud AA (2017). Biological Activity of *Moringa peregrina*: a Review. *American Journal of Food Science and Health*, 3, 83-87
35. Osman HE, Abohassan AA (2012). Morphological and analytical characterization of *Moringaperegrina* populations in Western Saudi Arabia. *International Journal of eoretical and Applied Sciences*, 4, 174-184
36. Rockwood JL, Anderson BG, Casamatta DA (2013). Potential uses of *Moringa oleifera* and an examination of antibiotic efficacy conferred by *M. oleifera* seed and leaf extracts using crude extraction techniques available to underserved indigenous populations. *International Journal of Phytotherapy Research*, 3, 61-71
37. Jung IL. Soluble extract from *Moringa oleifera* leaves with a new anticancer activity. *PloS one*. 2014; 9(4):e95492. doi: 10.1371/journal.pone.0095492 PMID: 24748376; PubMed Central PMCID: PMC3991666.
38. Al-Sharif I, R Emmal A, Aboussekhra A. Eugenol triggers apoptosis in breast cancer cells through E2F1/survivin down-regulation. *BMC cancer*. 2013; 13(1):600.
39. Sui L, Dong Y, Watanabe Y, Yamaguchi F, Hatano N, Tsukamoto I, et al. The inhibitory effect and possible mechanisms of D-allose on cancer cell proliferation. *International journal of oncology*. 2005; 27(4):907–12. PMID: 16142305.
40. Matsuda H, Ochi M, Nagatomo A, Yoshikawa M. Effects of allyl isothiocyanate from horseradish on several experimental gastric lesions in rats. *European journal of pharmacology*. 2007; 561(1):172–81.
41. Abdull Razis AF, Ibrahim MD, Kntayya SB. Health benefits of *Moringa oleifera*. *Asian Pacific journal of cancer prevention: APJCP*. 2014; 15(20):8571–6. PMID: 25374169.
42. Yamaguchi F, Takata M, Kamitori K, Nonaka M, Dong Y, Sui L, et al. Rare sugar D-allose induces specific up-regulation of TXNIP and subsequent G1 cell cycle arrest in hepatocellular carcinoma cells by stabilization of p27kip1. *International journal of oncology*. 2008; 32(2):377–85. PMID: 18202760
43. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. *Anticancer research*. 2001; 22(5):2587–90.
44. Al Kaabi SKS, Senthilkumar A, Kizhakkayil J, Alyafei MAM, Kurup SS, Al Dhaheri AS, Jaleel A. Anticancer Activity of *Moringa peregrina* (Forssk.) Fiori.: A Native Plant in Traditional Herbal Medicine of the United Arab Emirates. *Horticulturae*. 2022; 8(1):37. <https://doi.org/10.3390/horticulturae8010037>

TABLES AND RESPECTIVE CAPTIONS

Table 1A

DPPH radical scavenging activity of different *Moringa oleifera* extracts at different concentrations

Radical scavenging activities of extracts[#]							
Conc. (µg/100 µL)	Methanol	Ethanol 70%	Acetone	Chloroform	Ethyl acetate	n-Hexane	Conc. mean±SE
1000	92.54±0.05 ^a	93.94±0.07 ^a	97.62±0.09 ^a	82.54±0.06 ^a	91.10±0.02 ^a	94.59±0.08 ^a	92.05±4.70^a
500	91.89±0.05 ^a	93.40±0.05 ^a	84.92±0.06 ^b	68.27±0.07 ^b	78.43±0.01 ^b	51.40±0.06 ^b	78.05±14.61^b
250	91.24±0.02 ^a	93.24±0.03 ^a	77.46±0.04 ^c	60.22±0.05 ^c	71.94±0.06 ^c	43.40±0.07 ^c	72.92±17.33^c
125	82.86±0.04 ^b	86.81±0.04 ^b	71.24±0.03 ^d	57.08±0.06 ^d	56.64±0.05 ^d	36.22±0.05 ^d	65.14±17.29^d
65	69.89±0.03 ^c	71.51±0.05 ^c	66.27±0.03 ^e	56.38±0.07 ^d	46.81±0.03 ^e	10.16±0.04 ^e	53.50±21.16^e
Group mean±SE	85.68±8.65^b	87.78±8.54^a	79.50±11.00^c	64.90±9.78^e	68.98±15.69^d	47.15±27.46^f	

Table 1BDPPH radical scavenging activity of different *Moringa peregrina* extracts at different concentrations

Radical scavenging activities of extracts [#]							
Conc. (µg/100 µL)	Methanol	Ethanol 70%	Acetone	Chloroform	Ethyl acetate	n-Hexane	Conc. mean±SE
1000	96.32±0.09 ^a	98.49±0.23 ^a	96.43±0.67 ^a	87.57±0.37 ^a	91.24±0.26 ^a	87.13±0.33 ^a	92.86±6.35 ^a
500	95.89±0.06 ^a	97.62±0.56 ^a	96.27±0.75 ^a	77.94±0.38 ^b	83.13±0.22 ^b	75.13±0.45 ^b	87.66±8.85 ^b
250	95.78±0.05 ^a	96.65±0.45 ^a	89.99±0.35 ^b	77.72±0.39 ^b	78.49±0.47 ^c	69.57±0.47 ^c	84.70±10.75 ^c
125	94.81±0.04 ^a	95.10±0.20 ^a	82.32±0.58 ^c	75.89±0.44 ^b	71.62±0.44 ^d	68.81±0.45 ^c	81.43±15.95 ^d
65	93.94±0.09 ^a	94.32±0.05 ^a	76.38±0.37 ^d	68.60±0.33 ^c	69.40±0.70 ^d	65.57±0.57 ^d	78.40±17.69 ^e
Group mean±SE	95.35±6.75 ^a	96.44±7.65 ^a	88.28±10.65 ^b	77.54±12.89 ^c	78.78±11.85 ^c	73.24±14.25 ^d	

[#]Radical scavenging activity given as percentage inhibition

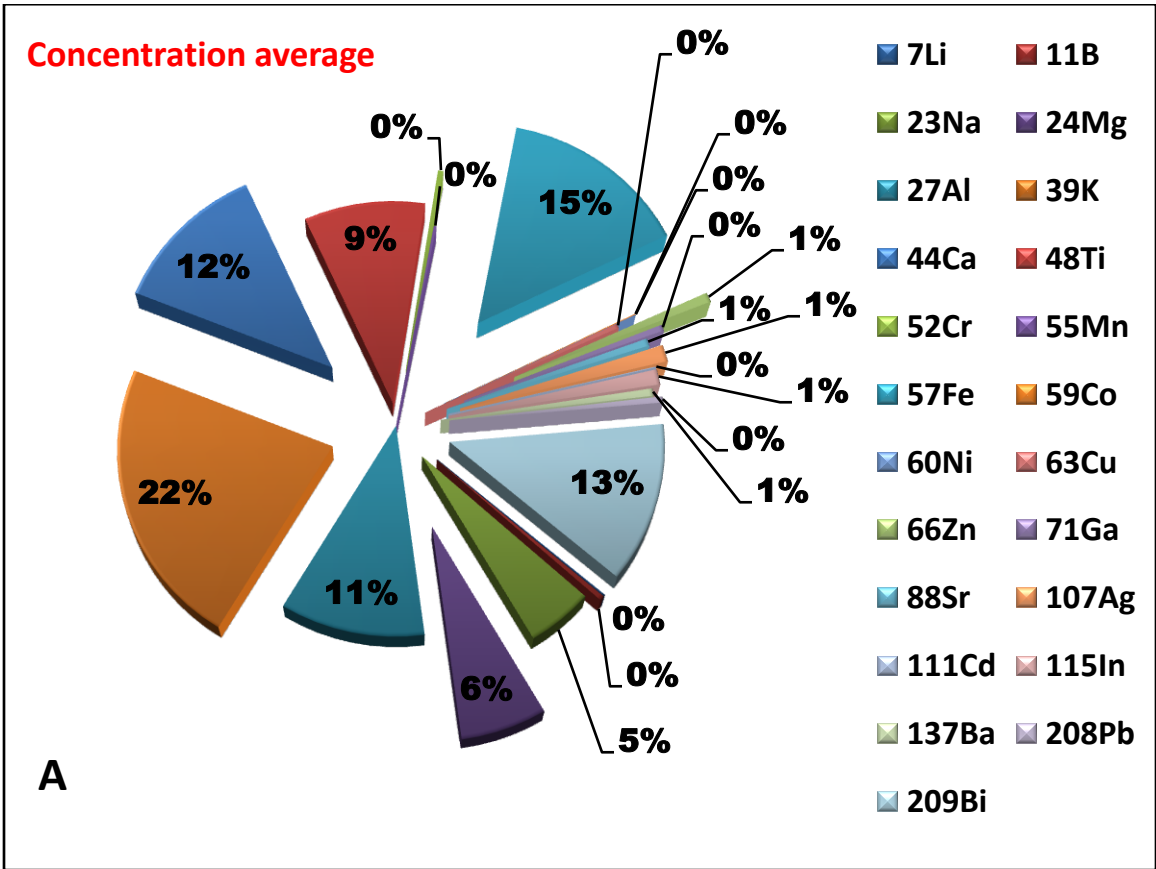
The percentage inhibition value of the standard compound ascorbic acid was 100%.

Values are means of three replicates and the relative standard deviations <1%

Means followed by the same letter(s) within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test**Table 2**The IC₅₀ values for extracts

Type of extract	IC ₅₀ (µg/ml)	
Methanol	107.4997 [#]	97.1378 [@]
Ethanol 70%	98.2137	214.3808
Acetone	2767.6654	166.3042
Chloroform	890.8855	651.4005
Ethyl acetate	58.2559	1132.6092
n-Hexane	4761.3347	4116.9485

[#]*Moringa oleifera*[@]*Moringa peregrina*The IC₅₀ values for *Moringa oleifera* extracts were calculated using Quest Graph™ IC₅₀ Calculator (AAT Bioquest, Inc, Sunnyvale, California, CA, USA). IC₅₀ was determined with a non-linear model



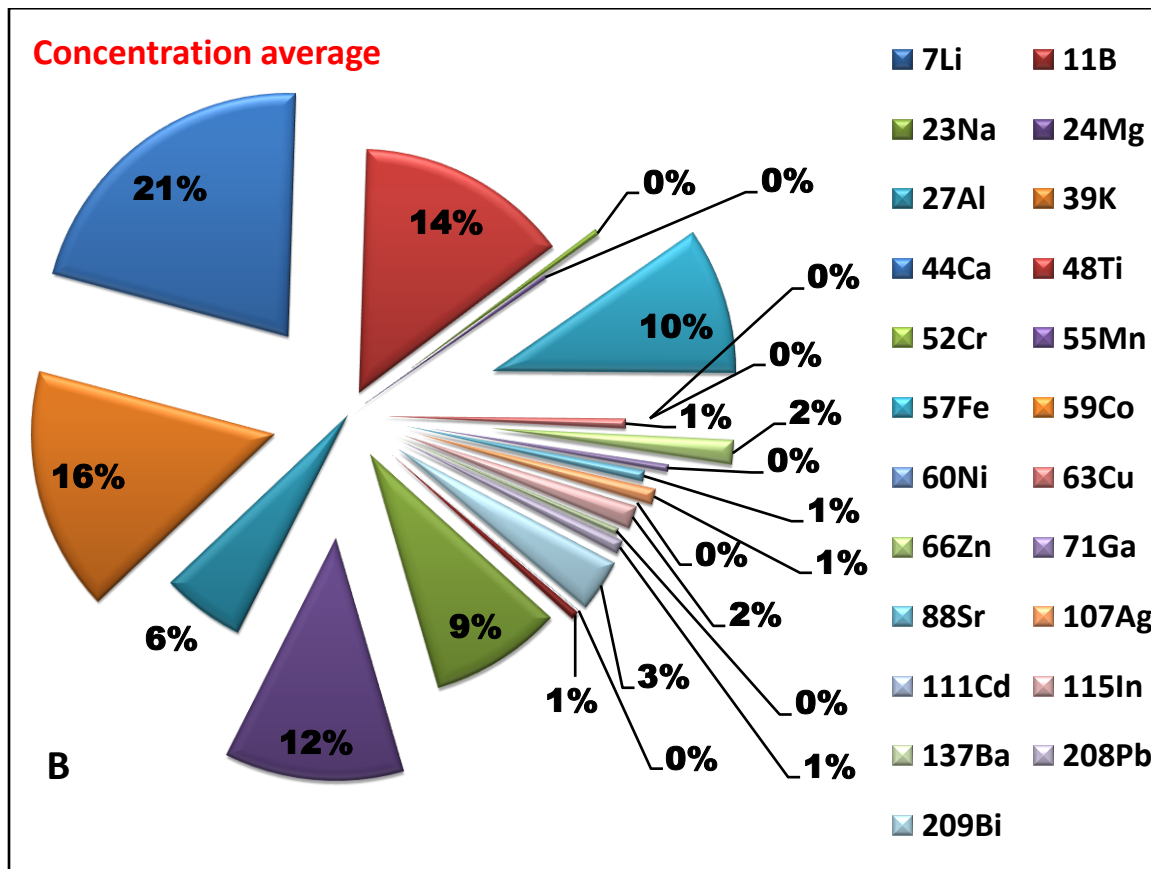
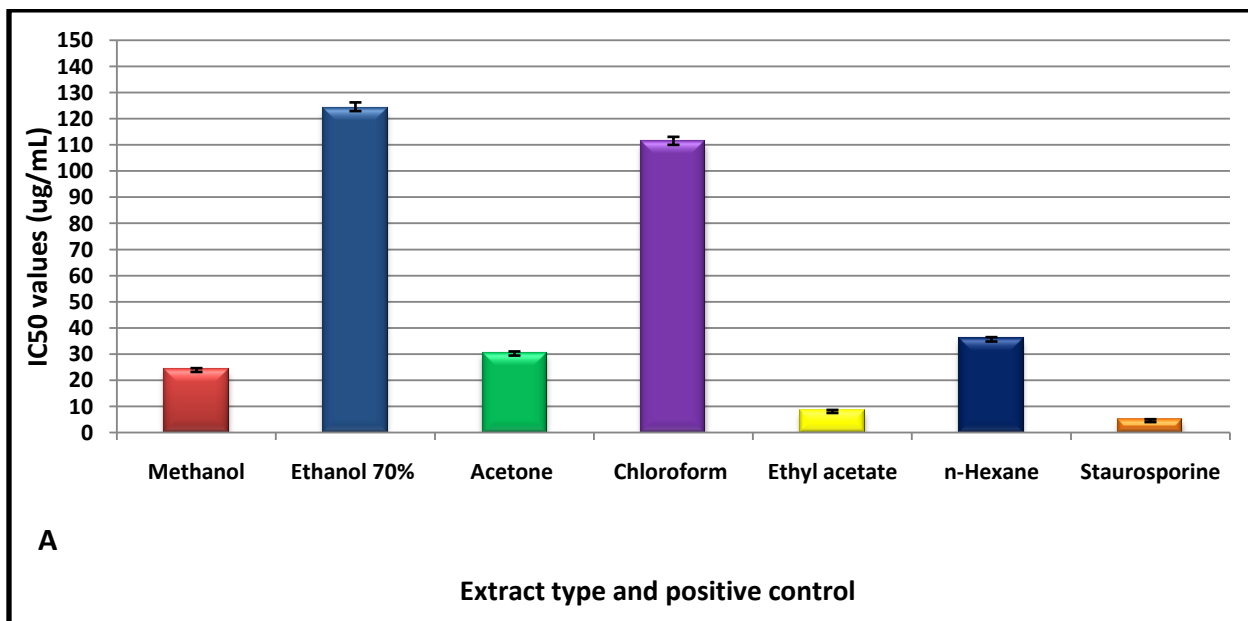


Figure 1
3D pie chart of minor and major elements of *Moringa oleifera* leaves (A) and *Moringa peregrina* leaves (B) (mg kg⁻¹ dry weight)



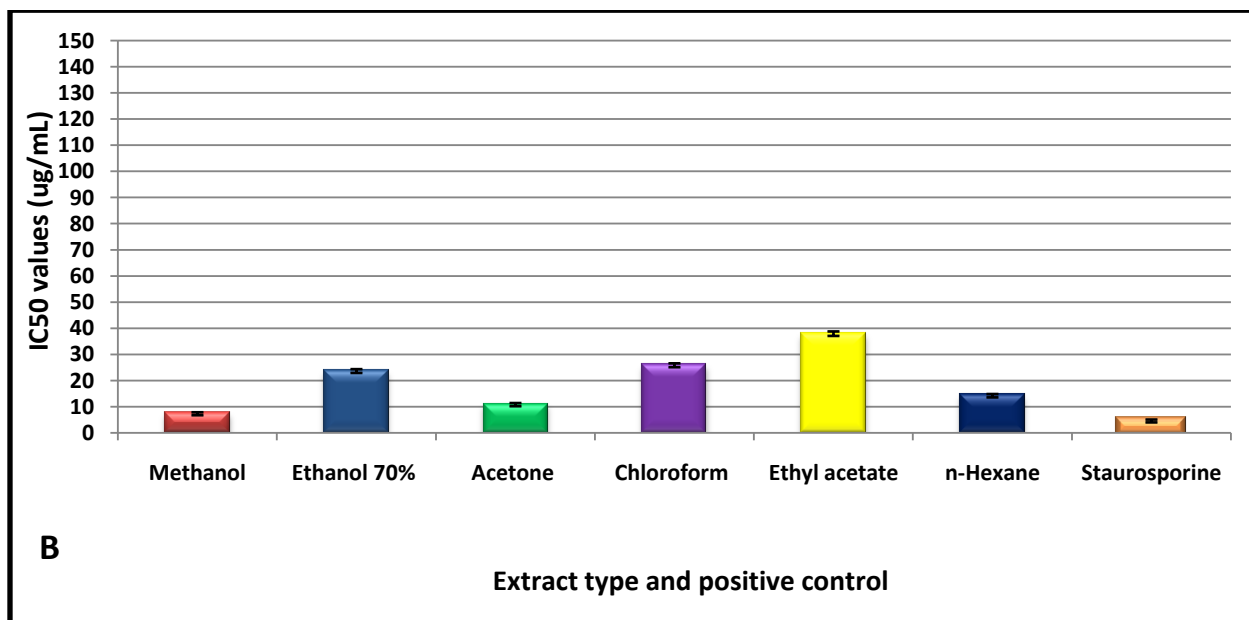


Figure 2
 IC₅₀ values of different extracts of Moringa species against human breast cancer cell line MDA-MB-231
2A, *Moringa oleifera*
2B, *Moringa pergerina*