Original Research Article

EVALUATION OF HYPOLIPIDEMIC POTENTIAL OF AQUEOUS SEED EXTRACT OF*MORINGA OLEIFERA* - AN *IN VITRO* STUDY

Abstract:

Introduction: Various parts of the tree Moringa oleifera Lam belonging to the Moringceae family are used extensively by the Indians as a major food constituent and also as herbal medicine. The dried seeds are used as a folk medicine as a hypolipidemic agent for patients who suffer from obesity.

Aim: The study was aimed at evaluation of hypolipidemic potential of aqueous seed extract of *Moringaoleifera* - an *in vitro* study

Materials and Methods: Hypolipidemic potential and antioxidant potential of aqueous seed extract of Moringa oleifera was analysed and compared with the standard drug.The data were analyzed statistically by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test and it was used to see the statistical significance among the groups. The results with the $p<0.05$ level were considered to be statistically significant.

Results: The DPPH radical scavenging activity showed that the plant extract possessed a significant in vitro antioxidant(Ic50=220µg/ml) and hypolipidemic activity.((Ic50=380µg/ml).

Conclusion: The aqueous seed extract of Moringa oleifera exhibited a significant antioxidant and hypolipidemic potential.

Key words: *Moringaoleifera*, seed extract, hypolipidemic activity, innovative technology, novel method.

Introduction:

Moringa oleifera, native to India, grows within the tropical and subtropical regions of the planet . It's commonly referred to as ‘drumstick tree’ or ‘horseradish tree’. Moringa can withstand both severe drought and mild frost conditions and hence it is widely cultivated across the planet. Having a high nutritive value, each and every part of the tree is suitable for either nutritional or commercial purposes. The leaves are rich in vitamins, minerals and other essential phytochemicals. It is used as a potential antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial agent. *M. oleifera* seed, a natural coagulant is extensively utilized in water treatment. (1,2)

Obesity has emerged as a serious ill health and risk factor for various disorders worldwide. Additionally to the present attenuation in adipogenesis and over expression of pancreatic lipase enzyme which plays a vital role in progression of obesity. Further, obesity has been found to be related to various disorders like osteoarthritis, ischemic heart diseases (IHD) , atherosclerosis, diabetes, and hypertension (1,2)A streak of evidence indicates that serotonin, histamine, dopamine, and their associated receptor activities are closely related to obesity regulation. Thus, attempts are made to scale back weight with such pharmacological intervention that possesses minimal side effects. Plants are used as traditional natural medicines for healing many diseases. Especially, various oriental medicinal plants are reported to possess biological activity. Literature review has revealed that various herbal plants like bladderwrack , sour orange, Green Tea, and Black Chinese Tea are utilized in the management of obesity.

*Moringaoleifera*Lam that belongs to Moringaceae family is usually referred to as golden shower tree that possesses various nutritional and medicinal values attributed to its roots, bark, leaves, flowers, fruits, and seeds. Recently, hypocholesterolemic activity of crude extract of M. oleifera crude extract was explored but it's thermogenic and antiobesity activity has not been investigated . Lopez et al has investigated the antiobesity property of methanolic extract M. oleifera leaves in experimentally induced obesity in experimental animals.(3)

Cardiovascular disease is the leading cause for death in India also as in western countries. Hyperlipidemia is one of the major causes for the development of cardiovascular disorder. In India, the leaves of Moringa oleifera Lam. is claimed to possess cholesterol-reducing effect and is employed to treat patients with heart disease and obesity.(4)The aqueous extract of the leaves

of M. oleifera was found to have wound healing and anti urolithiasis activity. (5)The methanolic crude extract of Moringaoleifera shows antibacterial activity.

In many cultures, herbal remedies are increasingly being employed to realize the medicinal value of the herbal plants.Our team has extensive knowledge and research experience that has translate into high quality publications (6),(7),(8),(9),(10),(11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24), (25). The current work exhibits the antioxidant and hypolipidemic potential of aqueous seed extracts of Moringa oleifera

Materials and methods:

1. Phytochemical Screening test

Test for phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

Test for Carbohydrates

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

Test for Flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

Test for Alkaloids

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

Test for Terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H2SO4 was added. Red color ppt obtained indicates the presence of terpenoids.

Test for proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

Detection of saponins

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

Test for steroids

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

2. **DPPH free radical scavenging activity of aqueous seed extract of Moringaoleifera**

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical was assessed by the method of Hatanoet al, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

DPPH radical scavenging (%) = Control OD-Sample OD X100

Control OD

3. ***In vitro*** **anti-cholesterol activity of aqueous seed extract of Moringaoleifera**

The anti-cholesterol assay was carried out as described as per the kit method (Spinreact, S.A.U-Ctra Santa Coloma, Girona, Spain). Cholesterol was dissolved in chloroform at a concentration of 2.5 mg mL/ml. Ten microliter of the extract was pipetted into microtiter plate followed by the addition of 2000 µL of R1 reagent and 10 µL of cholesterol as a sample. Twenty microliters of distilled water and 2000 µL of R1 reagents were used as blank. Negative control comprised of 20 µL cholesterol and 2ml R1; standard comprised of 20 µLsimvastatin and 2000 mL R1 reagent. The contents were incubated between 0-30 min at room temperature and the absorbance was read at 500 nm in a UV-Vis spectrophotometer against reagent blank. Anti-cholesterol assay of the extract was calculated using the following equation:

Inhibition (%) = Negative control-Sample× 100

Negative control

4. **Statistical Analysis**

The data were subjected to statistical analysis using one – way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of $p<0.05$.

Results and Discussion:

Table 1: List of phytochemicals and their extract

PHYTOCHEMICALS	EXTRACT
Protein	+
Amino Acids	+ + +
Flavonoids	+
Alkaloids	-
Terpenoids	+ +
Steroids	+
Saponins	+

Figure 1: *In vitro* antioxidant activity of aqueous seed extract of *Moringaoleifera*

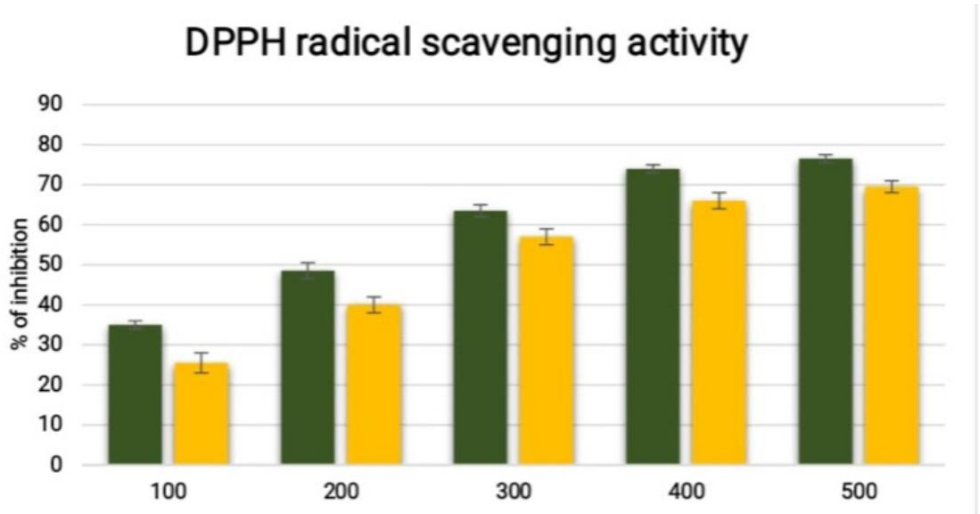

Figure 1 : Bar graph depicts the In vitro antioxidant activity of aqueous seed extract of *Moringaoleifera.* The X axis represents the different concentrations of *Moringaoleifera*seed extract taken and the Y axis represents the percentage of inhibition. Green colour denotes Vitamin C and the Yellow colour denotes *Moringaoleifera*seed extract. The difference was statistically significant. Each line represents Mean ± SEM of 3 independent observations. Significance at $p \leq 0.05$.

Figure 2: *In vitro* anti-cholesterol activity of aqueous seed extract of *Moringaoleifera*

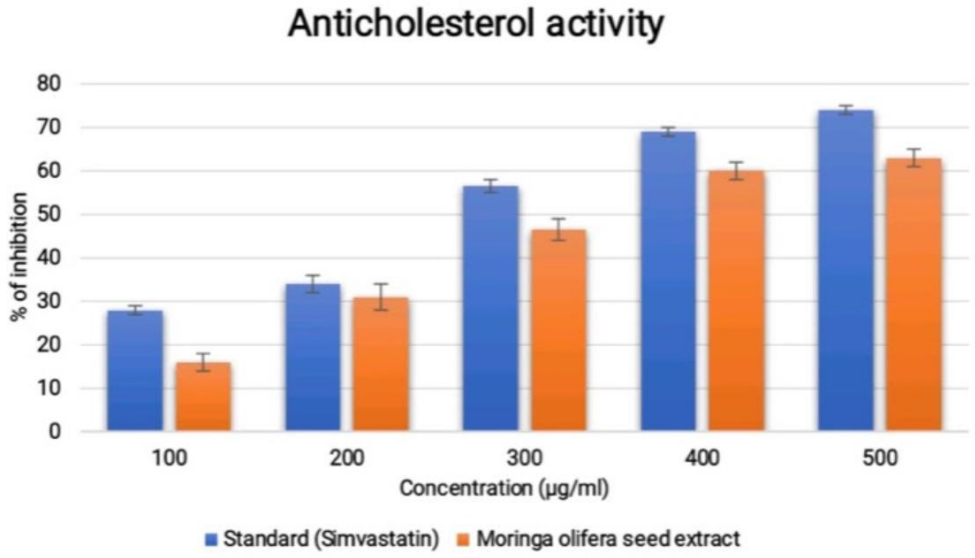

Figure 2 : Bar graph depicts the In vitro anti cholesterol activity of aqueous seed extract of *Moringaoleifera.* The X axis represents the different concentrations of *Moringaoleifera*seed extract and the Y axis represents the percentage of inhibition. Blue colour denotes the concentration of the standard drug Simvastatin and Orange colour denotes the *Moringaoleifera*seed extract. The difference was statistically significant. Each line represents Mean ± SEM of 3 independent observations. Significance at $p< 0.05$.

The qualitative phytochemical analysis of Moringa oleifera seed extract strongly showed the presence of proteins, amino acids, flavonoids, terpenoids, steroids, saponins.(Table 1). Phytochemical screening refers to the identification of medicinally active substances that are found abundant in plants. (26)Aqueous seed extract of *Moringaoleifera*showed in vitro antioxidant activity in a concentration dependent manner. Vitamin C is used as the standard drug for checking the antioxidant activity. Free radicals are reactive molecules in many physiological processes and have been associated with many diseases such as cancer. As a result there is a need to explore substances with free radical scavenging and antioxidant activity.(26,27) Every part of M. oleifera may be a storehouse of important nutrients and antioxidants.(28)The leaves of *Moringaoleifera*are rich in minerals like calcium, potassium, zinc, iron and copper. Vitamins like beta-carotene of vitamin A , B-complex vitamins like vitamin Bc , pyridoxine and niacin , vitamin C, D and E also present in Moringa oleifera. Phytochemicals like tannins, steroids, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugar present alongside anticancer agents like glucosinolates, isothiocyanates, glycoside compounds and glycerol-1-9-octadecanoate.(29)Moringa leaves even have a low calorific value and can be utilized in the diet of the obese. The pods are fibrous and are valuable to treat digestive problems and thwart carcinoma. A study done on Moringa shows that immature pods contain around 46.78% fiber and around 20.66% protein content. Pods have 30% of amino alkanoic acid content, the leaves have 44% and flowers have 31%. The immature pods and flowers showed similar amounts of palmitic, linolenic, linoleic and oleic acids.(30)

Moringa has a lot of minerals that are essential for growth and development among which calcium is taken into account together with the important minerals for human growth. While 8 ounces of spinach can provide 300–400 mg, moringa leaves can provide 1000 mg and moringa powder can provide quite 4000 mg. Moringa powder is often used as a substitute for iron tablets,

hence as a treatment for anemia. It's been reported that moringa contains more iron than spinach. An honest dietary intake of zinc is important for correct growth of sperm cells and is additionally necessary for the synthesis of DNA and RNA. M. oleifera leaves show around 25.5–31.03 mg of zinc/kg, which is that the daily requirement of zinc within the diet.(28,31,32)

Antioxidant activity of the seed extract was found to (Ic 50= 280µg/ml)increase in a dose dependent manner as compared to the standard(Vitamin C). The strong antioxidant property can be due to the rich phytoconstituents the seed possesses.(Fig 1)

The seed extract also exhibited a strong and significant hypolipidemic potential (Ic 50= 350µg/ml) as compared to the standard drug statin.(Fig 2)

Hypolipidemia may be a common disorder affecting about 2–3% of apparently healthy individuals. It would be a marker for an underlying, significant issue . Unexplained hyperlipidemia should be investigated for a possible cause. Several clinical conditions also as lipid lowering drugs may end in clinically significant hypolipidemia. Previous studies suggest that low cholesterol levels may function as a prognostic indicator in cancer patients.(33).Hypocholesterolemia is additionally a predisposing factor for infection in certain conditions also as a prognostic indicator during sepsis. there's a positive relationship between low total serum cholesterol levels, and increased mortality from all causes particularly in critically ill patients. Hyperlipidemia may predispose the critically ill patient to sepsis and adrenal failure and should carry a significantly increased risk of mortality. Currently, as we specialise in aggressive management of hyperlipidemia we should always keep an eye fixed on the possible complications of drug-induced hyperlipidemia.(34)

Conclusion:

Thus, from the present study it can be concluded that aqueous seed extract of *Moringaoleifera*showed potent in vitro antioxidant activity which was evident from the DPPH radical scavenging assay. A dose dependent anti cholesterol activity was observed for the extract and the standard drug statin. In the present study, the standard drug statin showed greater activity compared to the extract in all the tested concentrations.

NOTE:

The study highlights the efficacy of "herbal",”traditional” which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

References:

1. Milla PG, Peñalver R, Nieto G. Health Benefits of Uses and Applications of Moringa oleifera in Bakery Products [Internet]. Vol. 10, Plants. 2021. p. 318. Available from: http://dx.doi.org/10.3390/plants10020318

2. Bais S, Singh GS, Sharma R. Antiobesity and Hypolipidemic Activity of Moringa oleifera Leaves against High Fat Diet-Induced Obesity in Rats [Internet]. Vol. 2014, Advances in Biology. 2014. p. 1–9. Available from: http://dx.doi.org/10.1155/2014/162914

3. López SE, Pazos A, Gil A, Crespo J, Vargas C. Morphometry of fruit and seed of Moringa oleífera Lam. “moringa” [Internet]. Vol. 21, SCIÉNDO. 2018. p. 201–4. Available from: http://dx.doi.org/10.17268/sciendo.2018.020

4. Iswari RS, Mubarok I, Sasi FA. The Potential of Cnidoscolus chayamansa Alchoholic Leaves Extract as Hypolipidemia Agent [Internet]. Vol. 12, Biosaintifika: Journal of Biology & Biology Education. 2020. p. 83–9. Available from: http://dx.doi.org/10.15294/biosaintifika.v12i1.23687

5. Khan W, Parveen R, Chester K, Parveen S, Ahmad S. Hypoglycemic Potential of Aqueous Extract of Moringa oleifera Leaf and In Vivo GC-MS Metabolomics [Internet]. Vol. 8, Frontiers in Pharmacology. 2017. Available from: http://dx.doi.org/10.3389/fphar.2017.00577

6. Wu F, Zhu J, Li G, Wang J, Veeraraghavan VP, Krishna Mohan S, et al. Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on melanoma cells (B16). Artif Cells Nanomed Biotechnol. 2019 Dec;47(1):3297–305.

7. Chen F, Tang Y, Sun Y, Veeraraghavan VP, Mohan SK, Cui C. 6-shogaol, a active constiuents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating NrF2 signaling in human epidermal keratinocytes (HaCaT cells). J Photochem Photobiol B. 2019 Aug;197:111518.

8. Li Z, Veeraraghavan VP, Mohan SK, Bolla SR, Lakshmanan H, Kumaran S, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway [Internet]. Vol. 203, Journal of Photochemistry and Photobiology B: Biology. 2020. p. 111773. Available from: http://dx.doi.org/10.1016/j.jphotobiol.2019.111773

9. Babu S, Jayaraman S. An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. Biomed Pharmacother. 2020 Nov;131:110702.

10. Malaikolundhan H, Mookkan G, Krishnamoorthi G, Matheswaran N, Alsawalha M, Veeraraghavan VP, et al. Anticarcinogenic effect of gold nanoparticles synthesized from Albizia lebbeck on HCT-116 colon cancer cell lines. Artif Cells Nanomed Biotechnol. 2020 Dec;48(1):1206–13.

11. Han X, Jiang X, Guo L, Wang Y, Veeraraghavan VP, Krishna Mohan S, et al. Anticarcinogenic potential of gold nanoparticles synthesized from Trichosanthes kirilowii in colon cancer cells through the induction of apoptotic pathway. Artif Cells Nanomed Biotechnol. 2019 Dec;47(1):3577–84.

12. Gothai S, Muniandy K, Gnanaraj C, Ibrahim IAA, Shahzad N, Al-Ghamdi SS, et al. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. Biomed Pharmacother. 2018 Nov;107:1514–22.

13. Veeraraghavan VP, Hussain S, Balakrishna JP, Dhawale L, Kullappan M, Ambrose JM, et al. A Comprehensive and Critical Review on Ethnopharmacological Importance of Desert Truffles: Terfezia claveryi, Terfezia boudieri, and Tirmania nivea [Internet]. Food Reviews International. 2021. p. 1–20. Available from: http://dx.doi.org/10.1080/87559129.2021.1889581

14. Sathya S, Ragul V, Veeraraghavan VP, Singh L, Niyas Ahamed MI. An in vitro study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads. Environmental Nanotechnology, Monitoring & Management. 2020 Dec 1;14:100333.

15. Yang Z, Pu M, Dong X, Ji F, Priya Veeraraghavan V, Yang H. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway via attenuating the development of gastric carcinoma: In vitroandin vivostudies. Arabian Journal of Chemistry. 2020 May 1;13(5):5501–16.

16. Rajendran P, Alzahrani AM, Rengarajan T, Veeraraghavan VP, Krishna Mohan S. Consumption of reused vegetable oil intensifies BRCA1 mutations. Crit Rev Food Sci Nutr. 2020 Oct 27;1–8.

17. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021 Jun;126:105132.

18. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021 Mar;31(2):285–6.

19. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent. 2021 May;31(3):436–41.

20. Tang Y, Rajendran P, Veeraraghavan VP, Hussain S, Balakrishna JP, Chinnathambi A, et

al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from Scutellaria baicalensis on human osteoblast-like MG-63 cells [Internet]. Vol. 119, Materials Science and Engineering: C. 2021. p. 111656. Available from: http://dx.doi.org/10.1016/j.msec.2020.111656

21. Yin Z, Yang Y, Guo T, Veeraraghavan VP, Wang X. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway. Environ Toxicol. 2021 Jun;36(6):1011–20.

22. Veeraraghavan VP, Periadurai ND, Karunakaran T, Hussain S, Surapaneni KM, Jiao X. Green synthesis of silver nanoparticles from aqueous extract of Scutellaria barbata and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). Saudi J Biol Sci. 2021 Jul;28(7):3633–40.

23. Mickymaray S, Alfaiz FA, Paramasivam A, Veeraraghavan VP, Periadurai ND, Surapaneni KM, et al. Rhaponticin suppresses osteosarcoma through the inhibition of PI3K-Akt-mTOR pathway. Saudi J Biol Sci. 2021 Jul;28(7):3641–9.

24. Teja KV, Ramesh S. Is a filled lateral canal – A sign of superiority? [Internet]. Vol. 15, Journal of Dental Sciences. 2020. p. 562–3. Available from: http://dx.doi.org/10.1016/j.jds.2020.02.009

25. Theertha M, Sanju S, Priya VV, Jain P, Varma PK, Mony U. Innate lymphoid cells: Potent early mediators of the host immune response during sepsis. Cell Mol Immunol. 2020 Oct;17(10):1114–6.

26. Hamoudi M, Amroun D, Boutefnouchet S, Bensouici C, Kaoula S, Harzallah D, et al. Phytochemical screening, in vitro antioxidant and enzyme inhibitory proprieties and acute toxicity of extracts from the aerial parts of Ephedra nebrodensis, source of bioactive compounds. Comb Chem High Throughput Screen [Internet]. 2021 Mar 2; Available from: http://dx.doi.org/10.2174/1386207324666210303094339

27. Olayinka JN, Ozolua RI, Akhigbemen AM. Phytochemical screening of aqueous leaf extract of Blighia sapida K.D. Koenig (Sapindaceae) and its analgesic property in mice. J Ethnopharmacol. 2021 Feb 27;273:113977.

28. Habtemariam S. Investigation into the antioxidant and antidiabetic potential of Moringa stenopetala: identification of the active principles. Nat Prod Commun. 2015 Mar;10(3):475–8.

29. K E, Elumalai K. Antioxidant Activity and Phytochemical Screening of Different Solvent Extracts Cluasena excavata burm F. (Rutaceae) [Internet]. Vol. 1, Antioxidant Activity and Phytochemical Screening of Different Solvent Extracts Cluasena excavata burm F. (Rutaceae). 2016. Available from: http://dx.doi.org/10.15406/mojes.2016.01.00001

30. Oyewole I, Taiwo A, Quadri O. Evaluation of Hypoglycemic Efficacy of Methanolic Extracts of Moringa Oleifera and Phyllanthus amarus in Diabetic Rats [Internet]. Vol. 5, British Biotechnology Journal. 2015. p. 98–102. Available from:

http://dx.doi.org/10.9734/bbj/2015/13164

31. Abdulrazak M, Salim MA, El-ta'alu AB. Hypoglycemic effects of leaf extracts of Moringa oleifera and Vitex simplicifolia on blood glucose level of Wistar rat [Internet]. Vol. 2, Pyramid Journal of Medicine. 2020. Available from: http://dx.doi.org/10.4081/pjm.2019.36

32. Habtemariam S. The Pharmacology of Moringa stenopetala —Potential Anticancer Effects [Internet]. The African and Arabian Moringa Species. 2017. p. 143–54. Available from: http://dx.doi.org/10.1016/b978-0-08-102286-3.00010-5

33. Elmehdawi RR. Hypolipidemia and Sepsis: It Is the Hypolipidemia Not the Statins [Internet]. Vol. 3, Libyan Journal of Medicine. 2008. p. 65–65. Available from: http://dx.doi.org/10.4176/080508

34. Chemello K, García-Nafría J, Gallo A, Martín C, Lambert G, Blom D. Lipoprotein Metabolism in Familial Hypercholesterolemia. J Lipid Res. 2021 Mar 3;100062.