International Journal of Pharmaceutical Sciences and Drug Analysis

E-ISSN: 2788-9254
P-ISSN: 2788-9246
IJPSDA 2021; 1(1): 51-57
Received: 22-01-2021
Accepted: 24-03-2021

Azhagu Madhavan S
PG & Research Department of Zoology & Biotechnology, A. Veeriya Vandayar Memorial Sri Pushpam College, (Autonomous) Poondi, Thanjavur, Tamil Nadu, India

Correspondence
Azhagu Madhavan S
PG & Research Department of Zoology & Biotechnology, A. Veeriya Vandayar Memorial Sri Pushpam College, (Autonomous) Poondi, Thanjavur, Tamil Nadu, India

Pharmacological constituents and GC–MS analysis of bioactive compounds present in methanol leaf extract *Moringa oleifera*

Azhagu Madhavan S

Abstract
Moringa oleifera leaves, bioactive compounds within are may possess cancer-selective anti-proliferative properties. Previous research has been conducted in regards to this topic, but poor experimental design due to lack of necessary controls limits the legitimacy of anticancer claims. In order for anticancer claims to be sufficient and yield the opportunity of a potential cancer treatment, *Moringa* leaf extract must not harm non-cancerous cells. GC-MS analysis revealed the presence of major bioactive compounds 13 compounds with peak percentage in chromatogram. The major found were bioactive compound 1,2,3-Cyclopentanetriol R.T. (13.03), Tetradecanoic acid R.T. (18.67), 2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester R.T. (23.46). *M. oliefera* contains enormous bioactive compounds which have various biological activities. Therefore, it is recommended that newer *Moringa oleifera* extract based modern drug to be formulated and produce on commercial scale. *M. oleifera* in the prevention and treatment of a chronic diseases including inflammatory diseases, neuro-dysfunctional diseases, diabetes, and cancers its potential application in the prevention and treatment of chronic diseases or health encouragement.

Keywords: anticancer, diabetes, phytochemical analysis, GC-MS, *Moringa oleifera*

1. Introduction
The world are natural plant resources of a variety of biochemical products, many of which are extractable and find use in a number of pharmaceutical compounds. *Moringa oleifera* is a hardy tree with small, oval-shaped leaves and a thin trunk. It grows in dry places and hanging from its branches are large pods containing young seeds [1-3]. *Moringa* is a tree of many uses, for its horseradish tree and drumstick tree in India, "Miracle Tree" has commonly been used. *Moringa* has been used in traditional medicine for centuries, dating back to being used by the ancient Romans and Greeks [4]. Ayurvedic medicine, one of the world's oldest holistic healing systems, claims that *Moringa* can prevent up to 300 diseases, and aside from preventative measures, its leaves are curative properties as well. The traditional uses of *Moringa* are great in number, for they include the treatment of bacterial, fungal, viral, and parasitic issues, along with asthma, circulatory, digestive, and inflammatory disorders [5-7]. Other targeted ailments include malaria, typhoid fever, arthritis, hypertension, and diabetes. Because of *Moringa's* ability to improve the immune system, treatment of HIV and AIDS symptoms is also possible [8]. *M. oleifera* is rich in a wide range of secondary metabolites including proteins, vitamins, b-carotene, amino acids and various phenolics as flavonoids and phenolic acids Medicinally, various parts of *M. oleifera* have been widely employed as cardiac and circulatory stimulants, antitumor, antiepileptic, diuretic, antihypertensive, cholesterol lowering, hepatoprotective, antioxidant, antibacterial and antifungal agents [9]. All parts of *Moringa* have been utilized, for even the seeds are capable of purifying water. With this vast list of traditional medicinal properties, it comes as no surprise that *Moringa* is packed with chemical components to give it an astounding phytochemistry [10]. This is an insignificant claim; if healthy cells are killed or damaged at the same rate, *Moringa* leaf extract has no potential of possibly being developed into a cancer drug [11]. Also, controls must be conducted regarding the extract solvent used [12]. The solvent without leaf extract must be applied to the cells to affirm that the solvent is not producing the anticancer effect. Another aspect to consider determining the possibility of *Moringa* as a cancer treatment is the type of cell death that results, whether its apoptosis or necrosis. Apoptotic cells undergo shrinking, nuclear condensation, and disconnect from neigh boring cells or extracellular matrix. Immune defense consists of a non-specific (natural) immune system and a specific

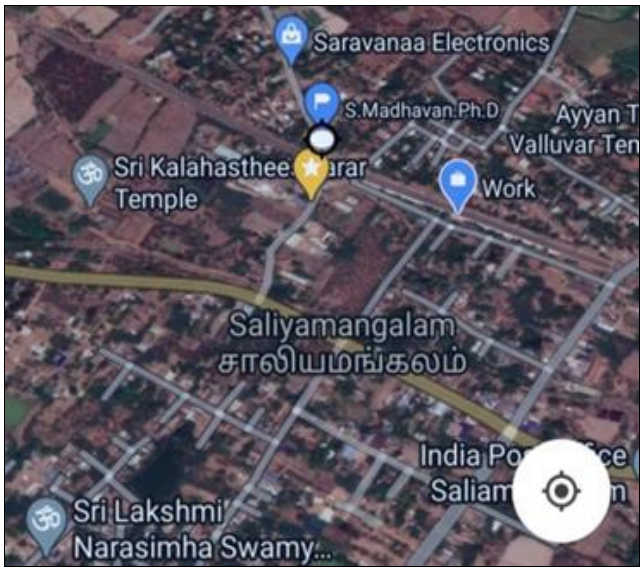
(adaptive) immune system [13]. *In-vitro* antioxidant assays with percentage of inhibition as a parameter are presented. Achieving this sort of cell death, the outside environment is left unaffected. Necrosis, however, is considered to be an "accidental" cell death that is uncontrollable and lacks order. When cancer cells undergo necrosis, the cell membrane is disrupted in a way that results in the intracellular components being released into the surrounding cellular environment. This release of material will lead to an inflammatory response by immune cells, which could possibly lead to further tumor growth.

2. Material methods

2.1 Plant Collection

The fresh leaves of *Moringa oleifera* were collected from Saliyamangalam, Thanjavur District, Tamil Nadu, India.

Fig: 1: *Moringa oleifer.*



Map 1: Study area

2.2 Plant material

The *Moringa oleifera* leaf was dried up under shade, crude powder. The crude type of the medication was utilize for the declaration of physicochemical boundaries similar to dampness content, debris esteems, increasing case, frothing evidence, unfamiliar natural issue, extractive qualities, and fluorescence analysis.

2.3 Phytochemical Studies

Moringa oleifera Secondary metabolites in the present studies were presence of medicinally active constituents. Beneficial drugs and to improve the patient health.

2.4 Preparation of extracts

The powdered plant samples of leaves (100 g) were used for successive solvent extraction (500ml) with increasing order of polarities like ethanol, methanol. At that point it is kept in an orbital shaker at 190-220rpm for 48 hours. The supernatant was collected, filtered through what man No.1filter paper and the extract were concentrated by a Rotary flask evaporator at a specific temperature was used based on the solvent system. Each time previous to extract through the next solvent the remains was dried thoroughly to remove the solvent used. The acquire dried up concentrate was then specifically gauged, put away in little vials at – 20 °C and utilized for the supplementary examinations.

2.5 Phytochemical screening

The preliminary phytochemical evaluation was carried out by using standard procedure [29].

2.6 Gas Chromatography-Mass spectrometry (GC-MS) analysis

Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column was used for GCMS analysis. Initially, the instrument was set to temperature of 110 °C, and then maintained at the same temperature for 2 min. At the end of this period, the oven temperature was raised upto 280 °C, at the rate of an increase of 5 °C per minute and maintained for 9 min. The temperature of injection port was ensured as 250 °C and the flow rate of Helium as 1 ml/min. The ionization voltage was 70 eV. The samples were injected regularly in opening mode as 10:1. The collection of mass spectrum was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The discontinuity examples of mass spectra were contrasted and those put away in the spectrometer information base utilizing National Institute of Standards and Technology Mass Spectral information base (NIST-MS). The percentage of each constituent was calculated from relation peak area of each component in the chromatogram.

Identification of compounds

Translation of mass range of GC-MS was directed utilizing the information base of National Institute Standard and Technology (NIST) having in excess of 62,000 examples. The unknown component's spectrum was compared with the spectrum of the known components stored in the NIST library. The structure, name and sub-atomic load of the parts of the test materials was learned.

3. Results and Discussion

3.1 Preliminary phytochemical screening

The consequence of the starter phytochemical examination of this current investigation may offer assurance to its ethnomedicinal utilization. The free revolutionaries present in our body are answerable for the age of numerous

sicknesses. In medication, it is utilized in hypercholesterolemia, hyperglycaemia, cell reinforcement, anticancer, calming, and weight reduction among others [15-17]. It is likewise known to have antimicrobial properties. India is in all probability the best maker of remedial flavors on the planet.

Table 1: Qualitative analysis of Phytochemicals analysis *Moringa oleifera leaves* extract

S. No	Analysed Phytochemicals factor	Methanol	Ethanol
1.	Tannin	+	+
2.	Saponin	-	+
3.	Flavonoids	+	+
4.	Steroids	+	+
5.	Alkaloids	+	-
6.	Polyphenol	+	+

Indications: "+" means positive activity, "-" means negative activity

Each constituent assumes a significant part and lack of any one constituent may prompt unusual advancements in the body [18]. While previous research has shown that *Moringa* leaf extract has the potential to kill cancer cells, the research fails to demonstrate the effects of *Moringa* leaf extract on healthy cells [19-21]. While the component of activity controlled by tannins is by upsetting the worm's negative particle body surge into positive particles (protonization), which at that point pull in sure worm body proteins in the gastrointestinal parcel, accordingly disturbing the digestion and homeostasis of the worm's body.

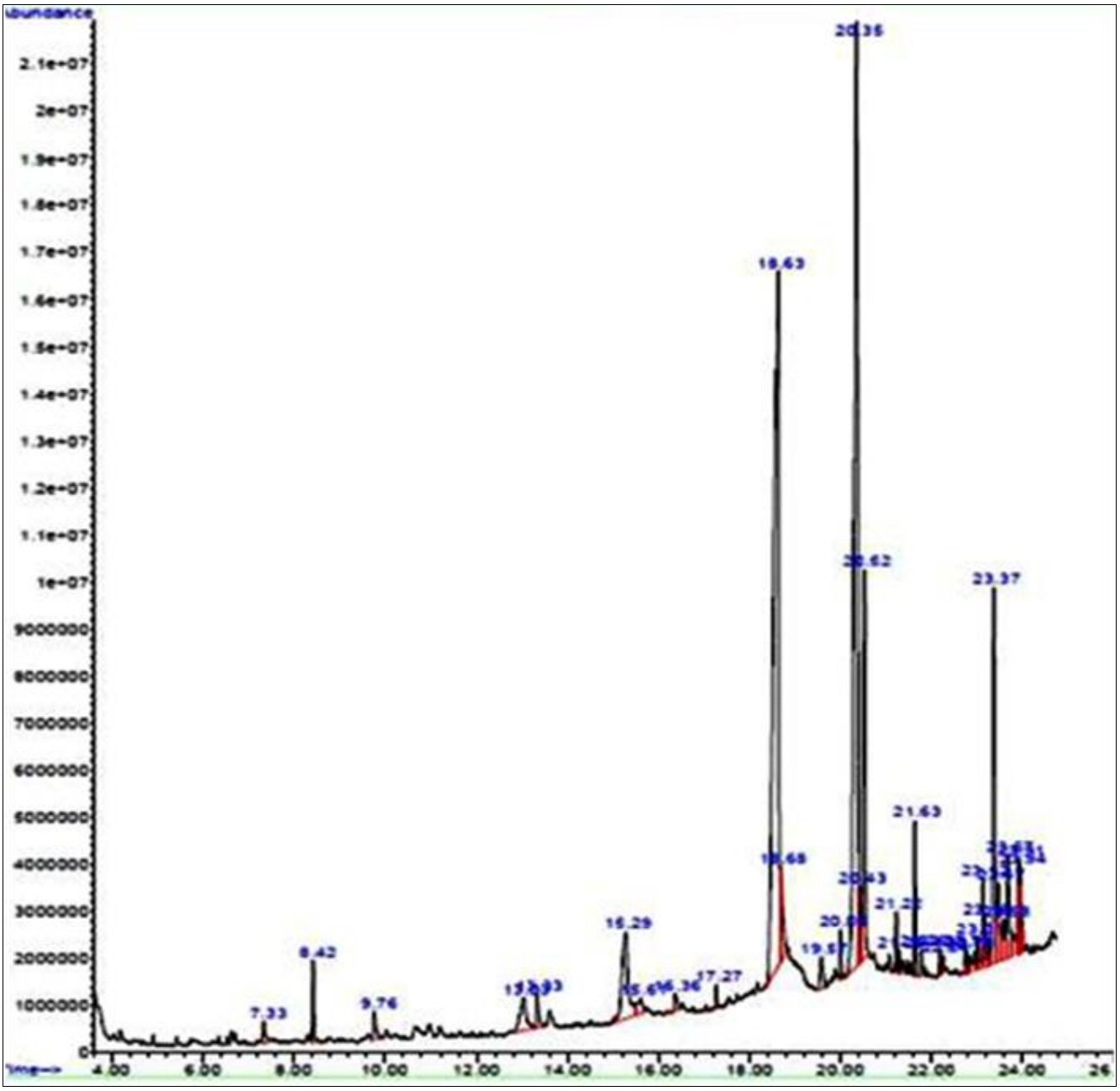
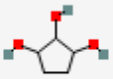
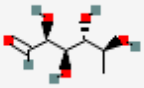
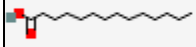
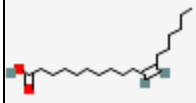
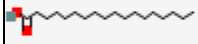
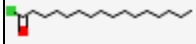
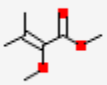
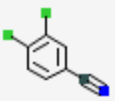
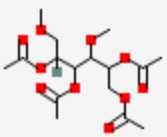


Fig 2: GC-MS chromatogram of *Moringa oleifera* Methanol *Leaves* Extract

Table 2: GCMS analysis - Bioactive compounds *Moringa oleifera* Methanol leaves extract

S. No.	Retention time	Compound name	Molecular formula	Molecular Weight	Area (%)	Structure
1	13.03	1,2,3-Cyclopentanetriol	$C_5H_{10}O_3$	118	1.68	
2	15.28	L-Galactose, 6-deoxy-	$C_6H_{12}O_5$	164	4.94	
3	18.67	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	2.18	
4	20.35	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	27.21	
5	20.52	Octadecanoic acid	$C_{18}H_{36}O_2$	284	4.82	
6	21.62	Palmitoyl chloride	$C_{16}H_{31}ClO$	274	1.51	
7	23.46	2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester	$C_7H_{12}O_3$	144	1.70	
8	23.67	3,4-Dichlorobenzonitrile	$C_7H_3Cl_2N$	172	2.28	
9	23.90	Mannitol,1,4-di-O-methyl-, tetraacetate	$C_{16}H_{26}O_{10}$	378	1.72	

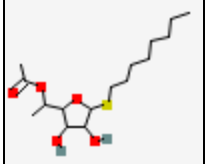
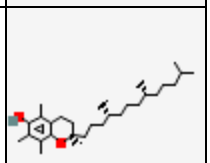
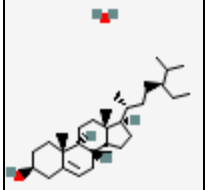
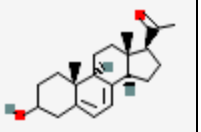
10	23.94	beta.-l-Rhamnofuranoside, thio-octyl-	$C_{16}H_{30}O_5S$	334	1.0	
11	27.85	Vitamin E	$C_{29}H_{50}O_2$	430	2.24	
12	29.16	gamma.-Sitosterol	$C_{29}H_{52}O_2$	432	2.82	
13	29.26	Pregn-5,7-diene-3-ol-20-one	$C_{21}H_{30}O_2$	314	1.42	

Table 3: GCMS analysis – Biological activity

S. No.	Retention time	Compound name	Molecular formula	Molecular Weight	Biological Activity
1	13.03	1,2,3-Cyclopentanetriol	$C_5H_{10}O_3$	**118**	Hypocholesterolemic, Anti-microbial
2	15.28	L-Galactose, 6-deoxy-	$C_6H_{12}O_5$	164	Flavoring agent
3	18.67	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	Antioxidant
4	20.35	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	Mild antioxidant and anti-atherosclerotic activity
5	20.52	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Protective against metabolic syndrome and cardiovascular disease risk factors
6	21.62	Palmitoyl chloride	$C_{16}H_{31}ClO$	274	Antioxidant, hypocholesterolemic nematicide, pesticide, flavor, lubricant, antiandrogenic, hemolytic 5-Alpha reductase inhibitor [22]
7	23.46	2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester	$C_7H_{12}O_3$	144	Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5- Alpha reductase inhibitor, anemiagenic insectifuge, flavor [23]
8	23.67	3,4-Dichlorobenzonitrile	$C_7H_3Cl_2N$	172	Anti-inflammatory
9	23.90	Mannitol,1,4-di-O-methyl-, tetra acetate	$C_{16}H_{26}O_{10}$	378	Anti-cancer
10	23.94	beta.-l-Rhamnofuranoside, thio-octyl-	$C_{16}H_{30}O_5S$	334	Antibacterial, Protective against metabolic syndrome and cardiovascular disease risk factors
11	27.85	Vitamin E	$C_{29}H_{50}O_2$	430	Antibacterial and Antifungal
12	29.16	gamma.-Sitosterol	$C_{29}H_{52}O_2$	432	Antimicrobial
13	29.26	Pregn-5,7-diene-3-ol-20-one	$C_{21}H_{30}O_2$	314	Antioxidant, hypocholesterolemic nematicide, pesticide,

The current investigation express that the presence of methyl or ethyl esters of unsaturated fats can likewise be considered as qualities of this plant. From this outcome, it very well may be rational to everyone the these mixes are of pharmacological significance as they have the properties, for example, antibacterial enemy of diabetic and pain relieving [24]. The non-specific immune response is the body's leading defense in the face of attacks by various foreign substances [25]. Cells that play a role in non-specific (natural) immune responses consist of phagocyte cells (macrophages and neutrophils) and NK cells (natural killer) [26]. Macrophages are phagocytic cells that play their function in the immune system by phagocytosis of foreign substances that enter the body. Macrophage response to microbes is almost as fast as neutrophils, but macrophages live longer than neutrophils. Macrophage phagocytosis is also more active in dealing with pathogens such as microorganisms or other antigens, and even cells or tissues themselves are damaged or dead so that macrophages can be categorized as primary effectors cells in the natural immune response.

4. Summary and Conclusion

Secondary metabolites phytochemical analysis presence of absence *In vitro* antioxidant, anti-cancer and anticoagulant activities to scientifically validate their folklore use in treatment of diseases [27-28]. This is a first- hand report that provides sufficient evidence for carrying out further

research on the selected plants to decipher the exact mechanism involved in anticancer and anticoagulant activity. Thereby suggesting *in vitro*, *in vivo* and secondary metabolite profiling studies to unravel and identify the bioactive compound(s) responsible, and ultimately provide alternative treatment strategies. GC-MS analysis major bioactive compounds and biological activity. Novel anticancer specialists are in effect effectively researched as existing treatments are creating opposition. Clinical oncologist utilize chemotherapeutic specialists that frequently affect of organs in spite of their adequacy against malignant growth cells. The viability of these medications is restricted bringing about administration of antagonistic medication responses, obstruction and conceivable treatment-related passing. The utilization of cytotoxic specialists in chemotherapy depends on the result from clinical preliminaries (Phase 1–3). Pharmacodynamics and pharmacokinetics assume an essential part for effective patient results. Along these lines, evaluate during the plan and improvement phase of elective medications. We have proposed the conceivable system for poly phenolic communication with gold metal particles for the phyto-nanoparticles development. To recognize the diminishing specialists present in the concentrate which caused the decrease Cancer is delegated one of the main sources of worldwide mortality. It has influenced a large number of individuals, regularly with helpless visualization. Having extreme results with regular chemotherapy, substitute medications and treatments are effectively being examined. There is a requirement for creative medication revelation and plan as existing malignancy treatments are exorbitant and not promptly accessible. Ayurveda and conventional medication have used regular assets like plants and trees as a component of their system to treat different ailment and sicknesses with positive results. One such tree is *Moringa oleifera*. In addition, these natural medicinal plants can be used for the synthesis of phyto-nanoparticles with targeted anticancer properties. With advancing medicine these agents can provide a source of easily accessible and affordable therapies in the future.

5. Acknowledgement
The author is also very grateful to the Secretary and Correspondent and the Principal of A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur (Dt), for providing the excellent infrastructure and necessary facilities to carry out my research work successfully.

6. Conflict of interest
The authors have declared that there is no conflict of interest.

7. Source/s of funding
No source of funding.

8. References
1. Pal S, Mondal S, Maity J, Mukherjee R. Synthesis, characterization of ZnO nanoparticles using *Moringa oleifera* leaf extract: investigation of photocatalytic and antibacterial activity, Int. J Nanosci. Nanotechnol 2018;14:111-119.
2. Tiloke C, Chuturgoon AA, The anti-proliferative and antibacterial effect of *Moringa oleifera*-mediated gold nanoparticles: a review, in: V.C. Kalia, A.K. Saini (Eds.), Metabolic Engineering for Bioactive Compounds - Strategies and Processes, Springer Nature Singapore Pte Ltd 2017, 269-291.
3. Abd-Rabou AA, Abdalla AM, Ali NA, Zoheir KM. *Moringa oleifera* root induces cancer apoptosis more effectively than leave nanocomposites and its free counterpart, Asian Pac. J Cancer Prev 2017;18:2141-2149.
4. Anand K, Tiloke C, Phulukdaree A, Ranjan B, Chuturgoon A, Singh S *et al*. Biosynthesis of palladium nanoparticles by using *Moringa oleifera* flower extract and their catalytic and biological properties, J Photochem. Photobiol. B 2016;165:87-95.
5. Moussa SAA, Abdel-Wahhab KG, Mannaa FA. *Moringa oleifera* inhibited the toxicity induced by administration of Tamoxifen in rats: using ultraviolet-visible and fluorescence spectroscopy investigations, J Inn. Pharm. Biol. Sci 2018;5:60-64.
6. Lin M, Zhang J, Chen X. Bioactive flavonoids in *Moringa oleifera* and their health promoting properties, J. Funct. Foods 2018;47:469-479.
7. Azhagu Madhavan* S. Phyto-pharmacological and GC-MS analysis of bioactive compounds presents in ethanolic extract *Solanum torvum* leaves. International Journal of Zoological and Entomological Letters 2021;1(1):32-37. [Cross Ref].
8. Matinise N, Fuku XG, Kaviyarasu K, Mayedwa N, Maaza M. ZnO nanoparticles via *Moringa oleifera* green synthesis: physical properties & mechanism of formation, Appl. Surf. Sci 2017;406:339-347.
9. Cheraghi M, Namdari M, Daraee H, Negahdari B. Cardioprotective effect of magnetic hydrogel nanocomposite loaded N,α-L-rhamnopyranosyl vincosamide isolated from Moringa oleifera leaves against doxorubicin-induced cardiac toxicity in rats: *in vitro* and *in vivo* studies, J. Microencapsul. 2017;34:335-341.
10. Ben-Arye E, Samuels N, Goldstein LH, Mutafoglu K, Omran S, Schiff E *et al*. Potential risks associated with traditional herbal medicine use in cancer care: A study of Middle Eastern oncology health care professionals, Cancer 2016;122:598-610.
11. Azhagu Madhavan S*. Priyadharshini R, Sripriya R, Uma V, Vinotha P. Pharmacognostical and Phytochemical Screening of GC–MS Analysis of Bioactive Compounds Present In Ethanolic Rhizome Extract of (*Zingiber officinale* Roscoe). J Biomed Res Environ Sci 2021;2(5):372-377. Doi: 10.37871/jbres1244, Article ID: JBRES1244. [Cross Ref].
12. Pangastuti A, Amin IF, Amin AZ, Amin M. Natural bioactive compound from *Moringa oleifera* against cancer based on in silico screening, J Teknol 2016;78:315-318.
13. Nishu Chandrawati Jee, Ravi Kumar. Gas Chromatography Study of Methanolic Leave Extract of *Moringa oleifera* Lam. Int. J Curr. Microbiol. App. Sci 2020;9(02):2590-2595.
Doi: https://doi.org/10.20546/ijcmas.2020.902.295.
14. Nweke Elizabeth Obioma, Akunne Wariso, Iheukwumere Chinedu Barry, Agu Esther. Evaluation of the potential of ethanol leaf extract of Persea americana in the treatment of on carbon tetrachloride (CCL4) induced renal damage. Int J Adv Biochem Res

2019;3(2):34-38.
DOI: 10.33545/26174693.2019.v3.i2a.102

15. Madi N, Dany M, Abdoun S, Usta J. *Moringa oleifera's* nutritious aqueous leaf extract has anticancerous effects by compromising mitochondrial viability in an ROS-dependent manner, J Am. Coll. Nutr 2016;35:604-613.
16. Silveira C, Shimabuku QL, Silva MF, Bergamasco R. Iron-oxide nanoparticles by the green synthesis method using *Moringa oleifera* leaf extract for fluoride removal, Environ. Technol 2017, 1-11.
17. Azhagu Madhavan* S. GC–MS Analysis of Bioactive Compounds Present In Ethanolic Leaf Extract *Acalypha indica*. Asian Journal of Advances in Research 2021;6(4):16-22.
18. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in *Moringa oleifera* leaves protect against chronic disease, Antioxidants 2017;6:1-13.
19. Gopalakrishnan L, Doriya K, Kumar DS. Moringa oleifera: a review on nutritive importance and its medicinalapplication, Food Sci. Hum. Wellness 2016;5:49-56.
20. Azhagu Madhavan* S, Priyadharshini R, Sripriya R. *In-vitro* pharmacological and GC-MS analysis of bioactive compounds presents in *Bytteneria herbaceae*. International Journal of Zoological and Entomological Letters 2021;1 (1):20-26.
21. Jaja-Chimedza BL, Graf Simmler C, Kim Y, Kuhn C, Pauli GF, Raskin I. Biochemical characterization and anti-inflammatory properties of an isothiocyanate enriched moringa (*Moringa oleifera*) seed extract, PLoS One 2017;12:1-21.
22. Sun A, Luo H, Deng L, Zhang S, Chen Z. The study on mechanism of the modified Chinese herbal compound, jianpijiedu, on a mouse model of hepatic carcinoma cachexia, Mol. Med. Rep 2016;14:3113-3121.
23. Olayinka LM, Clement B, Protective role of *Moringa oleifera* leaf-based diet on protein- energy malnutrition induced skeletal muscle degeneration, Int. J Sci. Rep 2017;3:54-62.
24. Sadek KM, Abouzed TK, Abouelkhair R, Nasr S. The chemo-prophylactic efficacy of an ethanol *Moringa oleifera* leaf extract against hepatocellular carcinoma in rats, Pharm. Biol 2017;55:1458-1466.
25. Guon TE, Chung HS. *Moringa oleifera* fruit induce apoptosis via reactive oxygen species-dependent activation of mitogen-activated protein kinases in human melanoma A2058 cells, Oncol. Lett 2017;14:1703-1710.
26. Monera-Penduka TG, Maponga CC, Wolfe AR, Wiesner L, Morse GD, Nhachi CFB. Effect of *Moringa oleifera* Lam. leaf powder on the pharmacokinetics of nevirapine in HIV-infected adults: a one sequence cross-over study, AIDS Res. Ther 2017;14:1-7.
27. Bhusnure OG, Jadhav PP, Hindole SS, Gholve SB, Giram PS, Kuthar VS. Green synthesis of silver nanoparticle using *Moringa oleifera* Lam extract for pharmacological activity, Int. J Pharm. Pharm. Res 2017;10:59-66.
28. Azhagu Madhavan* S, Ranjani R, Sripriya R. *In vitro* anti-oxidant activity of phytochemical analysis and various human cancerous cell line using microtiter plate based assay *Moringa oleifera* leaf extract. E-ISSN: 2788-8428. International Journal of Zoological and Entomological Letters 2021;1(1):38-43.
29. Azhagu Madhavan[a]* S, Vijayakumar S, Sripriya R, Rajalakshmi S. Phytochemical Screening and GC–MS Analysis of Bioactive Compounds Present in Ethanolic Leaf Extract *Murraya koenigii*. Bulletin of Environment Pharmacology and Life Sciences. Bull. Env. Pharmacol. Life Sci 2021;10(4):158-164. ISSN: 2277-1808.