Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS

Pharmaceutical Technology

Phytochemical Screening and *in-vitro* Antioxidant Properties of Synthesized Noni Seed Nanoparticles

Nnaoma, I. E[1*], Ezeibe Ndidi, F[2], Enete Uchenna, O[3], Joseph, R. C[1], Oguebie, R. N[1]

[1]Department of Pharmaceutical Technology, Federal Polytechnic Nekede Owerri, Federal Polytechnic Nekede along School Gate Nekede, 460113, Owerri, Nigeria
[2]Department of Hospitality Management Technology, Federal Polytechnic Nekede Owerri
[3]Department of Chemistry, Federal Polytechnic Nekede Owerri, Federal Polytechnic Nekede along School Gate Nekede, 460113, Owerri, Nigeria

DOI: 10.36347/sajp.2023.v12i04.001 | **Received:** 02.03.2023 | **Accepted:** 09.04.2023 | **Revised:** 17.04.2023 | **Published:** 23.04.2023

***Corresponding author:** Nnaoma, I. E
Department of Pharmaceutical Technology, Federal Polytechnic Nekede Owerri, Federal Polytechnic Nekede along School Gate Nekede, 460113, Owerri, Nigeria

Abstract **Original Research Article**

Nearly every facet of modern life has been profoundly impacted by the development of nanotechnology. Researchers from a variety of disciplinary domains are becoming interested in nanoparticles (NPs) with distinctive physicochemical features and broad medicinal uses. Because of their distinct structural characteristics, NPs have a wide range of uses in medication delivery, chemical and biological sensing, and other related fields. Oxidative processes are unavoidable as they are important in energy metabolism and the utilization of nutrients. The treatment available may not be sufficient to treat the disease as it is caused by many factors and there are so many adverse effects caused by the synthetic medicine available. The present study evaluated the phytochemical, phenolic, flavonoid, and antioxidant activity of Noni seed synthesized with silver nitrate. Fresh seeds of *Morinda citrofolia* obtained were extracted by maceration method and synthesis was done by reacting 10 mL of the *Morinda citrofolia* seed extract with 90 mL AgNO3 solution. Phytochemical, TPC, TFC, and antioxidant tests were carried out according to standard procedure. Results revealed that Noni seed AgNps contains alkaloids, tannins, flavonoids, resins, and terpenoids. Phenolic content was non-significantly different ($p>0.05$) and flavonoid content was significantly different ($p<0.05$) in comparison to gallic acid. Noni seed-AgNps extract also showed high antioxidant activity because of their higher percentage inhibitions very near to the controls used. From the study, we can conclude that Noni seed-AgNps extract has many important secondary metabolites and it has a potential for strong antioxidants.
Keywords: Noni seed, Medicinal Plants, Antioxidant, Phenols, Flavonoids.

INTRODUCTION

The development of nanoparticles in recent years had evolved various methods to synthesize nanoparticles of various sizes and shapes of metal. Metal nanoparticles possess unique chemical and physical properties from their bulk materials due to their high surface area to volume ratio (Murugan, and Natarajan, 2018). In recent medical applications, metal nanoparticle use has become standard. It has been observed that silver nanoparticles are extensively used for medical purposes due to their better chemical stability and good antimicrobial activity (Ruddaraju *et al.*, 2019). Research interest in nanotechnology has increased significantly due to the exponential nanomaterial production and marketing growth. Metallic nanoparticles can be shaped in different ways; spheres or rods. According to size; Nanomaterials are divided into different groups such as nanoparticles, dendrimers, nanotubes, and nanofilms. This further increases the variety of nanoscale materials (Han *et al.*, 2020).

The silver nanoparticles were well known for their antibacterial, and antioxidant property, and all other pharmacological potentials, which makes used for surgical prostheses and dental implants. In general, there are two approaches employed in the synthesis of nanoparticles, the first is the top-down approach (Physical methods), and the second is the bottom-down (chemical and biological methods) (Nakamura *et al.*, 2019). The physical method employs machining and etching to produce nanoscale structures whereas, other method employs chemicals such as sodium borohydride, ethanol, ethylene glycol, etc. as reducing agents (Ahmad, 2010). The chemical method is commonly used for producing silver particles. This is because of the simple equipment needed and the convenience to operate.

The use of herbal preparations as a form of therapy dates back many years ago. Their effectiveness in the general management of wellbeing has been attributed to the various phytochemical constituents commonly referred to as secondary metabolites. These compounds as used in phytomedicine can be considered to be the prime foundation of contemporary allopathic medicines today. The use and dependence on plants as medicines by man has been in existence since time immemorial and man continues to search for plants as a drug for a particular disease within his reach. Herbal medicines are safe than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway (Zaidan *et al.*, 2005). Plants are a good source of various functionally active secondary metabolites and a good source of essential nutrients (Khan *et al.,* 201). Screening active compounds from plants has led to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases including cancer and Alzheimers disease (Sheeja *et al*., 2007).

Oxidation is a process of manufacturing essential energy in living organisms. In that pathway of aerobic metabolism, distinctive reactive oxygen species (ROS) or reactive nitrogen species (RNS) are shaped as intermediates. ROS and RNS comprise free radicals that oxidize the membrane lipids. In this approach, one-of-a-kind cellular additives like proteins, lipids, nucleosides and nucleotides are damaged (Samad *et al.,* 2013). Hence, different sickness conditions appear likely, age-associated degenerative psychological disorders, atherosclerosis, cirrhosis, cancer, arthritis, diabetes, hemorrhagic shock, and so forth (Gülçin *et al*., 2010). Presently, there has been an increasing interest in the study of traditional plants and their medicinal value in different parts of the world. The medicinal properties of plants have been investigated in the light of recent scientific development throughout the world, due to their strong pharmacological activities, economic viability, and low toxicity (Prashant *et al*., 2010). Oxidative processes are unavoidable as they are important in energy metabolism and utilization of nutrients (Yakubu *et al*., 2019). Antioxidants help to scavenge free radicals and eliminate them from the body system. Studies have shown a retroverted relationship between disease progression and disease genesis and intake of antioxidant rich foods (Duduku *et al*., 2011). Although synthetic antioxidants are available, they are out of reach of many due to high cost, reduced distribution and side effects (Ravipati *et al*., 2012). An excess of ROS is also implicated in the genesis of many diseases such as cancer and other age-related diseases including inflammation (Diaz *et al*., 2012). This tremendous interest in plant-derived drugs is mainly due to the current widespread belief that herbal medicine is safer and more reliable than the costly orthodox medicine, many of which may have adverse side effects (Jigna *et al*., 2006). Nanoparticles are being employed in drug delivery systems because of their unique property. There are large numbers of physical, chemical, biological, and hybrid methods available to synthesize metal nanoparticles (Jyoti *et al*., 2016). Generally, physical and chemical methods are non-eco-friendly, toxic, and low in yield. The nanoparticles synthesized from chemical methods are medically non-applicable because of contamination from precursor chemicals (Jyoti *et al*., 2016).

Natural antioxidants are however very much available with minimal cost and showing little or no side effects. Compounds high in antioxidant ability are flavonoids and phenols. Trace elements such as copper, manganese and magnesium also act as antioxidants (Yakubu *et al*., 2019). In small concentrations, ROS are important in the human system as they help in gene expression, regulation of signal transduction and other biological processes (Yakubu *et al*., 2019). Since the ancient times, a great variety of plants have been used for therapeutic purposes. Most parts of plants have been used as extracts and may possess anti-inflammatory and antioxidant properties related to diseases such as diabetes, atherosclerosis, neurodegenerative, or cancer (Rodríguez-Yoldi, 2021). Natural compounds of plants have been utilized in the conventional system of medication to cure different infections and disorders. There are many plants whose total medicinal value is even now unknown and *Morinda citrofolia* is one among them. Screening active compounds from plants has led to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases.

A majority of the rich diversity of Nigerian medicinal plants is yet to be scientifically evaluated leading to its standardization and *Morinda citrofolia* is not left out. According to some study report, the optimum magnitudes of radical scavenging activity (RSA), and total phenolic content of Malaysian seedless *M. citrifolia* fruit methanol extract were 55.60% and 43.18 mg GAE/10 g, respectively (Krishnaiah *et al*., 2015). In another study, *M. citrifolia* anti-oxidant activity was evaluated as a natural anti-pigmentation agent by observing the effect of 50% ethanol extracts of Tahitian *M. citrifolia* fruit flesh, leaves, and seeds, on the tyrosinase enzyme responsible for controlling the production of melanin. The in vitro test was carried out using a tyrosinase inhibitory assay, showing that seed extract had stronger tyrosinase inhibitory (from 20 to 500 lg/ml) and antioxidant activity than the fruit (500 mg/ml), while leaf extract did not have any tyrosinase inhibitory activity at any concentration. The tyrosinase inhibitory activity was linked to the presence of lignans in *M. citrifolia*, particularly 3,30-bis demethylpinoresinol and americanin A (Masuda *et al*., 2009). The same seed extract was found to have anti-photoaging activity at concentrations ranging from 0.5 to 1.0 mg/ml (Matsuda *et al*., 2013), and also inhibited blood hemagglutination at concentrations ranging from 50 to 500 mg/ml (Matsuda *et al*., 2011); ursolic acid was the responsible chemical for both activities.

MATERIALS AND METHODS

Chemicals/reagents used

All the chemicals used in this study were of analytical grades and products.

Sample Collection and Preparation

Fresh seeds of *Morinda citrofolia* were collected from Uli in Anambra State of Nigeria and were identified and authenticated by a Taxonomist from the University of the Michael Okpara University of Agriculture Umudike. The seed was sun-dried for seven days and then ground into powder form to increase its surface area.

Extraction Method

During the extraction process, 500g of *Morinda citrofolia* seed powder was measured and soaked in 1000 ml of methanol for 72hrs. The mixture was filtered. The distillation process was applied to separate the solvent from the extract by evaporating to dryness. The stock solution of the extract was weighed and stored under refrigeration for further study.

Synthesis of Silver Nanoparticles (Ag-np)

The green synthesis of Ag-np was prepared following the method reported in the literature (Khan *et al*., 2018). Preparation was done by reacting 10 mL of the *Morinda citrofolia* seed extract with 90 mL AgNO3 solution (1 mM) and was agitated on the air bath magnetic stirrer for 15 minutes at room temperature. A color change was observed from colourless to pink. The mixture was centrifuged and dried in the oven at a temperature between 50°C – 60°C overnight.

Phytochemical Analysis

A small amount of the synthesized AgNPs with *M. citrofolia seed* extract was used for the phytochemical analysis. The phytochemical tests included the test for alkaloids, flavonoids, tannins, saponins, terpernoids, and resins adopting the approach of Ashika *et al*., (2018) and Patel & Vakilwala (2016).

Determination of Total Phenolic and Flavonoid Content

Total phenolic and flavonoid content of the *M.citrofolia* fruit was determined following the standard procedure of Baba and Malik (2015).

In-Vitro Antioxidant Assay

DPPH, and FRAP assay was carried out as described by Azam *et al*., (2015).

Statistical analysis

Statistical analysis of the data was carried out with Graph Pad Prism version 9.0 using t-test one paired test. The statistically analysed data was reported as significantly different at 95% confidence level of probability ($P < 0.05$).

RESULTS

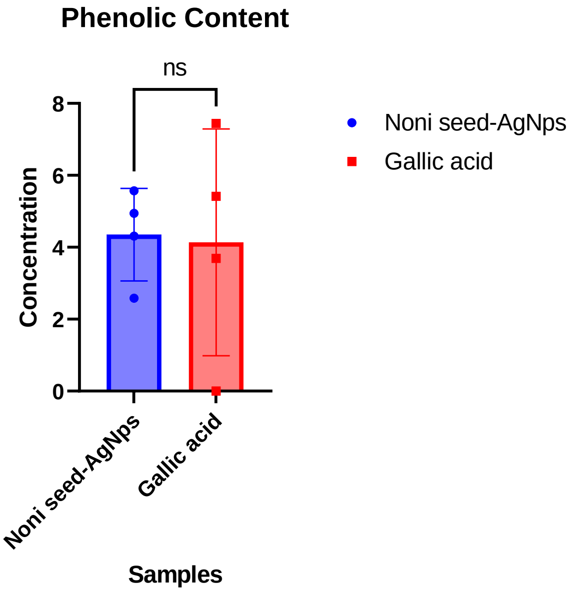

Figure 1: Graph above reveals the amount of phenols in Noni seed synthesized with silver nitrate. Phenol amount in Noni seed-AgNps compared to gallic acid standard were non-significantly different (P>0.05) from each other

Total Phenolic content

Concentration (mg/ml)	Noni seed-AgNps	Gallic acid
10	2.58	-
20	4.31	3.69
40	5.57	5.42
80	4.94	7.440

Total Flavonoid content

Concentration (mg/ml)	Noni seed-AgNps	Gallic acid
10	6.704	13.277
20	11.399	15.577
40	12.948	18.160
80	12.432	18.911

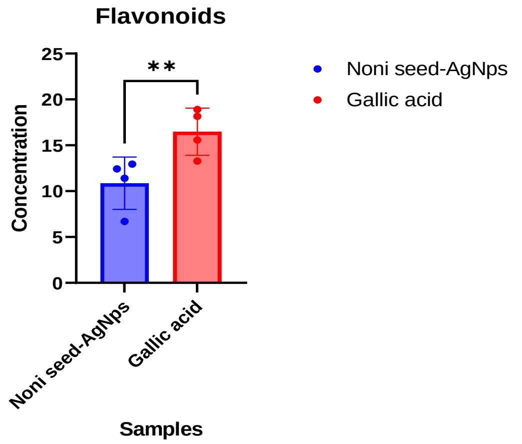

Figure 2: Graph above reveals the amount of flavonoids in Noni seed synthesized with Silver nitrate. Flavonoid amount in Noni seed-AgNps compared to gallic acid standard were significantly different (P<0.05) from each other

Ferric reducing antioxidant power (FRAP)

Concentration (mg/ml)	Noni seed-AgNps [% Inhibition]	Gallic acid [% Inhibition]
10	55.265	78.422
20	71.843	81.316
40	78.948	84.737
80	80.264	-

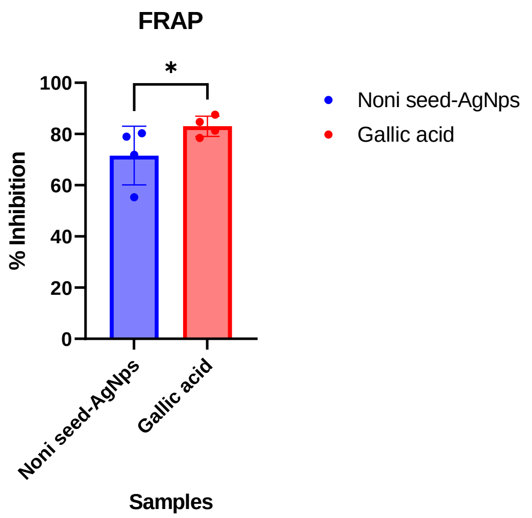

Figure 3: Result of the above FRAP analysis reveals that the % inhibitory activity of noni seed-AgNps in comparison to gallic acid standard was significantly different and lower (p<0.05). Hence, Noni seed-AgNps has considerable ability to inhibit and reduce the risk of oxidative stress

2,2-diphenyl-1-picrylhydrazyl (DPPH)

Concentration (mg/ml)	Noni seed-AgNps [% Inhibition]	BHT [% Inhibition]
10	90.57	98.999
20	88.62	
40	90.20	
80	88.46	

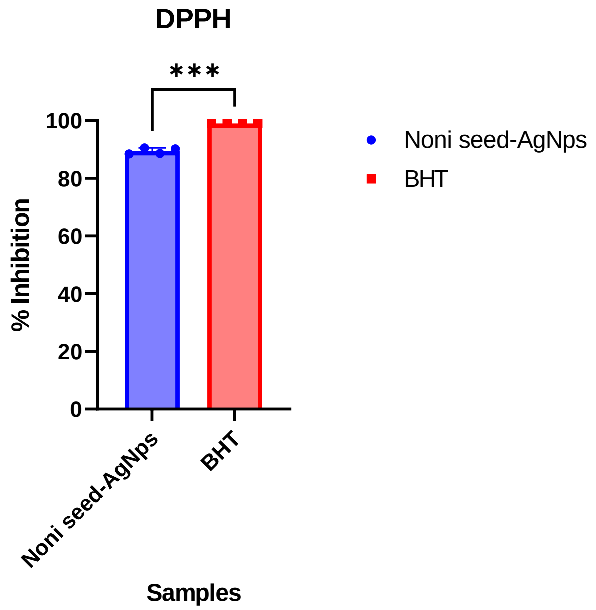

Figure 4: Result of the above DPPH analysis reveals that the % inhibitory activity of noni seed-AgNps in comparison to *Butylated hydroxytoluene (*BHT) standard was significantly different and lower (p<0.05). However, noni seed-AgNps exhibited antioxidant activity

Phytochemical test result of Noni seed-AgNps

Parameters	Noni seed-AgNps	
	Observation	Remark
Alkaloid Wegners reagent test Meyers reagent Test	++ reddish brown precipitation found ++ precipitation form	Alkaloid high high
Flavonoid Ferric chloride	++ black ppt form	high
Lead acetate test	++ precipitate form	high
Tannin acid test	++ red ppt form	high
Lead acetate method	++ black ppt form	high
Resin	+ light formation of emulsion	low
Saponin	+ light formation of emulsion	low
cardiac glycoside	+ brick red ppt formed	low
Terpenoid	+ light reddish brown ppt	low

DISCUSSION

Plant and plant-based products are the natural sources of different phytochemicals such as phenols, flavonoids, alkaloids, glycosides, lignins, and tannins. Phenols and flavonoids are the most common phytoconstituents of different fruits, vegetables, and medicinal and aromatic plants, which are responsible for antioxidant activities (Scalbert *et al*., 2005). Due to the potential toxicological effects of synthetic antioxidants natural antioxidants such as phenols and flavonoid compounds from plant origin are gaining popularity these days (Zhong *et al*., 2013). Antioxidants are tremendously important substances, which possess the ability to protect the body from damage caused by free radical-induced oxidative stress. Plant polyphenols act as reducing agents and antioxidants by the hydrogen-donating property of their hydroxyl groups.

Phytochemical result revealed the presence of Alkaloid, Tannin, Flavonoid in abundance and saponin, glycoside, terpenoids, resin in minute amount. Study result reveals that noni seed-AgNps contains an appreciable amount of phenols which was non-significantly different ($p>0.05$) from gallic acid standard. Flavonoid content in noni seed-AgNps was significantly different ($p<0.05$) from that of gallic acid. Phenolic content of any plants is directly related to their anti-oxidant properties. They act as reducing agents, hydrogen donors and are capable of scavenging free radicals (Wojdylo *et al*., 2007). Phenolic antioxidants are potent free radical terminator. The phenolic compounds, biologically active component are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid peroxidation at the first initiationstep (Babu *et al*., 2013). Flavonoids on the other hand, possess a broad spectrum of chemical and biological activities including radical scavenging properties. Collectively, flavonoids are of particular importance in the human diet as there is evidence that they act as antioxidants, antiviral and anti-inflammatory agents (Chioma *et al*., 2020) and are associated with reduced risk of cancer and cardiovascular diseases (Chioma *et al*., 2020). Kaempferol, quercetin and myricetin which are flavonoids reduced the risk of pancreatic cancer by 23 percent in an 8-year study (Chioma *et al*, 2020). The presence of flavonoids suggests that the plant might have anti-oxidant, anti-allergic, anti-inflammatory, anti-microbial, anti-cancer activity as confirmed by the study.

For antioxidant abilities, FRAP is a sensible and practicable indicator of total anti-oxidant capacity. The Ferric reducing power was determined using the FRAP assay. The results derived from the FRAP assay show the antioxidant potential in Noni seed-AgNps through the reduction of Ferric iron ($Fe3+$) to ferrous iron ($Fe2+$) by the anti-oxidants present in Noni seed-AgNps. The concentrations of the extract have a good inhibition compared to that of the control (Gallic's acid) and have good higher antioxidant capacity that is the percentage inhibition increases at higher concentration. During the reduction of the Ferric iron, a blue colour develops that is read colourimetrically at 594nm. DPPH is a kind of unstable free radical and accepts an electronic or hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate radical scavenging activity of Noni seed-AgNps (Blois 1958). The table shows that the extracts 10mg - 80mg (88%-90%) exhibited a good antioxidant activity compared to the control, BHT (98.99%). This finding shows that Noni seed-AgNps contains high antioxidant potential and can be used for the prevention of vascular disease, anti-aging and cancer.

CONCLUSION

The findings in this work shows that Noni seed-AgNps exhibits an active or good anti-oxidant potential or activity. This was possible because of the presence of high phenolics. The plant also showed inhibitory activity using FRAP and DPPH assay in dose-dependent way. Further studies or research should be carried out on the isolation and identification of the antioxidant of the extract and its function.

ACKNOWLEDGEMENT

The researchers wish to acknowledge TETfund for providing the fund used in the research, the Management of Federal Polytechnic Nekede and Directorate of Research and Development for providing the enabling environment.

Funding: The research was funded by Tertiary Education Trust Fund, TETfund Nigeria.

REFERENCES

- Ahmad, M. B. (2010). Synthesis and characterization of silver / talc nanocomposites using the wet chemical reduction method. *Int J Nanomedicine*, 5, 743–751, https://doi.org/10.2147/IJN.S13227
- Ashika, B. ., Roy, C. L., S, N., S, S. K., Suma, A., & Sathyamurthy, B. (2018). Phytochemical Studies on the Methanolic Extract of Coriander Sativum Leaves - An In Vitro Approach. *European of Biomedical and Pharmaceutical Sciences*, *5*(8), 494–500. http://www.ejbps.com
- Azam, S., Ansari, P., Rashid, M. M., Alam, M. N., Ahmed, I. H., Ibarahim, M. Y., Shafi, S. M., Rahman, S., & Hossen, A. (2015). In vitro anti-oxidant and in vivo anti-inflammatory activity determination of the methanolic leaves extract of millettiapachycarpa. *Biomedical Research and Therapy*, 2(10).
- Baba, S. A., & Shahid A. Malik, S. A. (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science*, 9, 449–454.
- Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1199–1200.
- Chioma, A., Albert, A., & Kenechukwu, A. (2020). Chemical Profile of the Stem Extract of Costus afer (Bush Cane) from Imo State in Nigeria. *Asian Journal Of Research In Biochemistry*, 113-123. doi: 10.9734/ajrb/2020/v7i430154
- Diaz Jr, L. A., Williams, R. T., Wu, J., Kinde, I., Hecht, J. R., Berlin, J., ... & Vogelstein, B. (2012). The molecular evolution of acquired resistance to

targeted EGFR blockade in colorectal cancers. *Nature*, *486*(7404), 537-540.

- Duduku, K., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species, *Food Bioprod. Process,* 89, 217-233.
- Gülçin, İ., Bursal, E., Şehitoğlu, M. H., Bilsel, M., & Gören, A. C. (2010). Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. *Food and Chemical Toxicology,* 48, 2227-2238.
- Jigma, P., & Sumitra, G. I. (2006). In-vitro antimicrobial activities of extracts of *Launea procumens* Roxb. (Labiateae) *Vitis vinitera* L. (Vitaceae) and *Cyperus rotundus* L. (Gyperaceae). *African Journal Biomedicine* 9, 89-93.
- Jyoti, K., Baunthiyal, M., & Singh, A. (2016). Characterization of silver nanoparticles synthesized using Urtica dioica Linn. leaves and their synergistic effects with antibiotics. *Journal of Radiation Research and Applied Sciences*, 9(3), 217–227. doi:10.1016/j.jrras.2015.10.002
- Khan, M. Z. H., Tareq, F. K., Hossen, M. A., & Roki, M. N. A. M. (2018). Green synthesis and characterization of silver nanoparticles using *Coriandrum sativum* leaf extract. *Journal of Engineering Science and Technology*, *13*(1), 158–166.
- Krishnaiah, D., Bono, A., Sarbatly, R., & Anisuzzaman, S. M. (2015). Antioxidant activity and total phenolic content of an isolated *Morinda citrifolia* l. methanolic extract from poly-ethersulphone (pes) membrane separator. *J King Saud University –Eng. Sci*, 27(1), 63–67.
- Masuda, M., Murata, K., Fukuhama, A., Naruto, S., Fujita, T., Uwaya, A., Isami, F., & Matsuda, H. (2009). Inhibitory effects of constituents of *Morinda citrifolia* seeds on elastase and tyrosinase. *J. Nat. Med*, 63(3), 267-273.
- Matsuda, H., Masuda, M., Murata, K., Abe, Y., & Uwaya, A. (2013). Study of the anti-photoaging effect of noni (*Morinda citrifolia*), Melanoma – From Early Detection to Treatment.
- Matsuda, M. M., Murata, K., Itoh, K., Naruto, S., Uwaya, A., & Isami, F. H. (2011). Effects of *Morinda citrifolia* extract and its constituents on blood fluidity. *J Tradit. Med*, 28(2), 47–54.
- Motshakeri, M., & Ghazali, H. M. (2015). Nutritional, phytochemical and commercial quality of noni fruit: A multi-beneficial gift from nature. *Trends in Food Science and Technology*, 45(1), 118–129.
- Murugan, N., & Natarajan, D. (2018). Bionanomedicine for antimicrobial therapy - a case study from Glycosmis pentaphylla plant mediated silver nanoparticles for control of multidrug resistant bacteria. *Lett. Appl. NanoBioScience*, 8, 523-540. https://doi.org/10.33263/LIANBS834.523540
- Nakamura, S., Sato, M., Sato, Y., Ando, N., Takayama, T., Fujita, M., & Ishihara, M. (2019). Synthesis and Application of Silver Nanoparticles (Ag NPs) for the Prevention of Infection in Healthcare Workers. *International Journal of Molecular Sciences*, 20, 135-140. https://doi.org/10.3390/ijms20153620
- Patel, K., & Vakilwala, M. (2016). Phytochemical study and bioactivity of solvent extracts on Coriandrum sativum. *International Journal of Advanced Research in Biological Sciences*, *3*(5), 193–199
- Prashant, K. R., Dolly, J., Singh, K. R., Guupta, K. R., & Watal, C. (2008). Glycemic properties of *Trichosanthes dioica* leaves. *Pharmarceutical Biology*, 46(12), 894-899.
- Rodríguez-Yoldi, M. J. (2021). Anti-Inflammatory and Antioxidant Properties of Plant Extracts. *Antioxidants*, 10, 921.
- Ruddaraju, L. K., Pallela, P. N. V. K., Pammi, S. V. N., Padavala, V. S., & Kolapalli, V. R. M. (2019). Synergetic antibacterial and anticarcinogenic effects of Annona squamosa leaf extract mediated silver nano particles. *Materials Science in Semiconductor Processing*, 100, 301-309. https://doi.org/10.1016/j.mssp.2019.05.007
- Scalbert, A, Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). "Dietary polyphenols and the prevention of diseases," *Critical Reviews in Food Science and Nutrition*, 45(4), 287–306.
- Wojdylo, A., Oszmianski, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs, *Food Chemistry,* 105(3), 940–949.
- Yakubu, O. F., Adebayo, A. H., Iweala, E. E. J., Adelani, I. B., Ishola, T. A., & Zhang, Y. (2019). Anti-inflammatory and antioxidant activities of fractions and compound from *Ricinodendron heudelotii* (Baill.). *Heliyon*, 5, 02779.
- Zaidan, M., Rain, N., & Badrul, A. (2005). In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Tropical Biomedical*, 22, 165-170.