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INVESTIGATING THE BIOCHEMICAL AND CYTOTOXIC POTENTIALS OF SUBMERGED FERMENTED EXTRACT OF *Moringa oleifera* LEAVES BY *Saccharomyces cerevisiae*

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ABSTRACT

Moringa oleifera leaves contain several phytochemicals that serve as excellent functional secondary metabolites. This *Moringa* leaf broth has been fermented using *Saccharomyces cerevisiae* in order to enhance the bioactive constituents that can exhibit improved biochemical and cytotoxic activities. The GC-MS result of fermented *Moringa* leaf broth showed the presence of a characteristic mix of metabolic compounds different from non-fermented broth. Comparatively, the fermented broth had a higher level of antioxidant activity (67.30 ± 0.34%) and ethanol (4.1 ± 0.09%) than the non-fermented sample.Furthermore, the fermented broth showed antibacterial activity against *Bacillus cereus*, *Klebsiella pneumoniae*, and *Salmonella typhi,* and anticancer activity MCF-7 cell line *in vitro* in a concentration-dependent manner. Finally, the liposomes encapsulating the fermented *Moringa* leaf broth were assessed for similar cytotoxic activities and proved to be significantly nearer to the values obtained for the Test (fermented) sample. Hence, the fermented *Moringa* leaf broth has improved properties and thus it can replace the synthetic products.

Keywords: *Moringa* Leaf Broth, Fermentation, *Saccharomyces cerevisiae*, Biochemical Properties, Antibacterial, Cytotoxic Activities, Liposomes.

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INTRODUCTION

Moringa leaves have tremendous phytochemicals like vitamins, carbohydrates, alkaloids, tannins, polyphenols, flavonoids, saponins, and isothiocyanates attributing to several medicinal and research benefits.[1] These bio-constituents of *Moringa* leaves can be enriched or value-added by implementing fermentation under optimal conditions.[2] It is best suited to perform Submerged Fermentation (SmF) of *Moringa* leaves so that, the microorganisms inoculated into the broth will be able to completely use the ingredients of the *Moringa* leaf broth.[3,4]Submerged Fermentation (SmF) employs the process of cultivation of microbes in liquid culture media that may contain submerged solid particles which also act as substrates. During fermentation, most of the complex organic compounds provided as nutrients are converted into simpler and bioactive metabolites by the immense activity of functional enzymes and other catalysts generated by the microorganisms.[5] As these microorganisms grow, they start utilizing the nutrients that are provided to them and release many byproducts.[6] All of these byproducts may be processed and tested for their desired properties such as antioxidant, antibacterial, anticancer, anti-inflammatory, etc.,[7,8]Fermented products which are plant-derivedwith the help of microorganisms are being extensively used in our daily lives. With the right nutrients and substrates, *Saccharomyces cerevisiae* (Baker's yeast) can perform its fermentation to an adequate extent.[9] In general, *S. cerevisiae* can be used in the fermentation of both alcoholic and non-alcoholic nutrients simultaneously which

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finally releases a variety of end metabolites possessing characteristic biological activities. Mostly, yeast-fermented plant broths contain end products like peptides, phenols, coumaric acid, and ferulic acid derivatives. [10] In this research, we have performed submerged fermentation of *Moringa* leaves using *Saccharomyces cerevisiae* at a pilot scale level. Thus the obtained fermented *Moringa* leaf broth was used to assess several of its biochemical and cytotoxic activities *in vitro* by comparing the test (fermented broth) and control (non-fermented broth) samples. To extend the study, the fermented *Moringa* leaf broth was encapsulated within the liposomes and characterized accordingly.

EXPERIMENTAL

Materials

2,2-Diphenyl-1-picrylhydrazyl (DPPH), (3-(4,5-Dimethylthiazol-2-yl)-,2,5-Diphenyltetrazolium Bromide) MTT, Glucose, Potassium Dichromate, Sodium Carbonate, Sodium Chloride and Tris-HCL supplied by HiMedia that were of Analytical Grade (AR) were used. Folin Phenol Reagent, Hydrochloric acid, and Sulphuric acid were purchased from Nice. Nutrient Broth and Agar from SRL were used for antimicrobial tests. Baker's yeast (*Saccharomyces cerevisiae*) was purchased from a nearby grocery store in Coimbatore, India.

Preparation of *Moringa* Leaf Broth and Fermentation

Moringa leaves were collected from a residential area near Eachanari, Coimbatore, India. The leaves were first cleaned thoroughly under flowing tap water and then with deionized water. The leaves were then subjected to air drying under aseptic conditions. To prepare *Moringa* leaf broths, 4 g of *Moringa* leaves were cut into small pieces, and Four grams of glucose were dissolved in 100 milliliters of deionized water. Likewise, two separate *Moringa* leaf broths were prepared and added into 250 mL conical flasks in which one served as the Control and the other served as the Test samples. Both the flasks containing *Moringa* leaf extract were sterilized by autoclaving at standard conditions. Fermentation was performed by adding 1% of activated baker's yeast only into the test sample and the flasks were kept incubated for five days at 37ºC. After 5 days, the control and test samples were passed through the Whatman filter for filtration, and the resulting filtrates were centrifuged for 15 minutes at 10,000 rpm. Subsequently, additional analyses were conducted using the supernatants from the control and test samples.

Total Phenol Content Estimation

0.5 mL of the test sample, control sample, and liposomal solution were each added to separate tubes. To each tube, 2 mL of ten-fold diluted Folin phenol reagent was added, and the contents were thoroughly mixed. Following this, 4 mL of a 7.5% disodium carbonate solution was added to all tubes and kept at room temperature for 30 minutes. [11] The optical densities of the samples were then measured at 750 nm using a colorimeter, with gallic acid used as a baseline for comparison and 0.5 mL of deionized water used as a blank. The trial was conducted in triplicates.

Estimation of Ethanol

The potassium dichromate method was used for estimating the ethanol content in the samples.[12]A modified protocol was adopted. 32.5 mL of concentrated sulfuric acid and 17.5 mL of distilled water were combined to create the potassium dichromate reagent. 3.4 g of potassium dichromate was then added to the acid solution. Individual tubes were filled with 1 mL of 1-5% standard ethanol solutions and 1 mL of deionized water was used as blank. The final volumes of the Control and Test samples were measured at 0.3 mL and were diluted to 1 mL using distilled water. To all the tubes, 1 mL of potassium dichromate reagent was added and incubated at 80°C in a hot water bath for 10 minutes. The optical densities were measured at 600nm for all the standards including the triplicate samples.

Antioxidant Activity

The antioxidant activity of non-fermented (Control) and fermented (Test) *Moringa* leaf broths was measured by DPPH assay.[13] The antioxidant activities of the samples were calculated as follows:

$$\text{Antioxidant Activity (\%)} = \frac{(\text{Negative Control Absorbance} - \text{Sample Absorbance})}{\text{Negative Control Absorbance}} \times 100$$

Synthesis of Liposomes Encapsulating the Fermented *Moringa* Leaf Broth

Liposomes were synthesized according to with slight modifications. 5mL of chloroform was used to dissolve Lecithin and Cholesterol in a 9:1 ratio, respectively, in a round-bottom flask. A thin film of lecithin:cholesterol mixture was achieved by removing the chloroform vapors in a rotary evaporator under vacuum conditions for 4 hours. The phospholipid film was hydrated by the addition of 40 mL of Fermented *Moringa* leaf broth and agitated for 1 hour. After that, the mixture was processed using a cooling centrifuge at 12,000 rpm. Ultimately, the liposomes that contained the fermented *Moringa* leaf broth were reconstituted in separate volumes of 2 mL of 0.9% NaCl solution (standard saline) and 5 mL of distilled water separately. The liposomal suspension in normal saline was used for antibacterial and cytotoxicity assays. Similarly, the liposomal suspension in distilled water was used in the characterization of the liposomes.

Characterization of the Liposomes

The particle size and zeta potential of the synthesized liposomes were calculated using Dynamic Light Scattering (DLS) using Malvern Zetasizer. The Zeta Runs parameter was 15 and the Measurement Position was 2.00 mm. The temperature was 25°C with a count rate of 456.3 kbps.

Antibacterial Properties

The antibacterial activity of the control and test *Moringa* leaf broths, along with the liposomes containing the test broth, was evaluated using the well diffusion technique. Three pathogenic strains namely *Bacillus cereus*, *Klebsiella pneumoniae,* and *Salmonella typhi* were used. Briefly, the bacteria were inoculated onto separate nutrient agar plates. Three wells were bored aseptically in the nutrient agar plates. To each plate, 50 µL of Control, Test, and liposomal suspension were added into individual wells. The plates were incubated at 37°C overnight and the diameter of the Zone of Inhibition (ZI) for each sample against the bacteria was measured and interpreted. Triplicates were maintained for all the samples and test bacteria.

Cytotoxicity Assay

The MCF-7 cell line was grown and maintained in DMEM media supplemented with 1% penicillin and 1% streptomycin added to it along with 10% FBS. 100 µL of the cultivated MCF-7 cells were planted into each well of a 96-well microtiter plate with fresh media, and the plate was then incubated at 37°C in a 5% CO_2 incubator for 24 hours. After incubation, five different increasing concentrations (2-10 µL) of fermented *Moringa* leaf broth (Test sample) and liposomal suspension containing the fermented broth were added into individual wells.[14]10 µL of normal saline was substituted for Test samples as a Control. The microtiter plate was incubated for another 24 hours at the same conditions. Finally, the media were removed from all the wells, and the cells were washed with normal saline. Then, each well-containing cell received 20 µL of MTT reagent. The volume was made up to 100 µL using normal saline. After a 4hours of incubation, the absorbance of the plate was measured by employing an ELISA reader at 570 nm.

Statistical Analyses

The statistical analyses for all the triplicates were performed using Microsoft Excel 2010. In this article, all findings are presented as "mean ± standard error of the mean," and were the notable statistical result ($p \leq 0.05$) and consistent with the null hypothesis.

RESULTS AND DISCUSSION

GC-MS Analysis

The chromatogram obtained (Fig.-1) for fermented *Moringa* leaf broth confirmed the presence of most of the non-polar active metabolites in the samples. The metabolic constituents are relatively high in fermented *Moringa* leaf broth. The retention time, area, and the respective compounds analyzed by GC-MS are tabulated in Table-1. All of the identified compounds in GC-MS are hypothesized to have one or more biological activities both *in vitro* and *in vivo* that are quantitatively estimated in the following assays. To discuss, about eleven compounds were detected by GC-MS in the ethanolic leaf extract of *Moringaoleifera* with a total yield of around 62.87% which was higher than the aqueous extract.[15] Out of these eleven compounds already identified and reported, compounds namely have also been detected in our research along with other compounds. Again, 25 peaks were obtained after analyzing the methanolic

leaf extract of *Moringa* with similar compounds. However, we were able to identify a few other compounds in the chloroform extract of *Moringa* leaves. According to previously published research, the aqueous extract of fermented *Moringa* leaves produced by a different yeast strain contained 31 components.

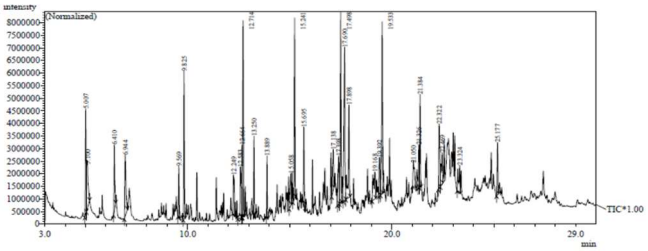

Fig.-1: GC Chromatograph of Fermented *Moringa*Leaf Extract

Table-1: Phytochemical Constituents of Fermented *Moringa*Leaf Extract

S. No	Elution Time(in minutes)	Constituent Name	Molecular Formula	Relative Pertcentage
1	5.007	Phenol	C6H5OH	4.05
2	5.100	Nonane	C9H20	3.71
3	6.410	Hexane,3,3-dimethyl-	C8H18	2.36
4	6.944	Phenylethyl alcohol	C8H100	3.38
5	9.569	Undecane,2,4-dimethyl-	C13H28	1.70
6	9.825	Octane, 5-ethyl-2-methyl-	C11H24	4.50
7	12.249	Decane, 3,7-dimethyl-	C12H26	2.07
8	12.583	Tetradecane, 4-ethyl-	C16H34	2.99
9	12.664	Phenol, 3,5-bis(1,1-dimethylethyl)-	C14H22	2.22
10	12.714	Hexadecane	C16H43	6.56
11	13.250	Tridecane, 4-methyl-	C14H30	2.21
12	13.889	Dodecane	C12H26	1.63
13	15.695	Dodecane, 4-methyl-	C13H28	2.57
14	17.398	2-Bromotetradecane	C14H29	2.72
15	17.498	Eicosane	C20H42	7.29
16	17.690	Pentadecanoic acid	C15H30O2	8.10
17	19.168	Heneicosane	C21H44	2.83
18	19.392	Octacosane	C28H58	1.93
19	19.533	Triacontane, 1-iodo-	C30H61	7.69
20	21.326	Nonacosane	C29H60	1.66
21	21.384	Tetratetracontane	C44H90	2.72
22	22.322	Hexadecanoic acid, 2-hydroxy-1-(hydroxyl	C19H3804	3.07
23	25.177	Squalene	C30H50	1.95

Total Phenol Content Estimation

The total phenol contents were estimated for both control and test samples. The control sample which is made up of only aqueous *Moringa* leaf extract and the test sample, made up of fermented *Moringa* leaf broth contain the same phenolic content of about. The amount of phenols was estimated to be 1.058 mg of Gallic Acid equivalents per gram of dry weight. Also, fermentation of plant leaves could sometimes increase the phenolic content of the broth which has been experimentally quantified to be 25.28 mgGAE/g after the fermentation of *Orthosiphonst amineus* leaves by *S. cerevisiae*.[16]

Ethanol Estimation

Yeast fermentation in general, releases ethanol as the most prominent byproduct by consuming sugars supplanted in the broth. The Non-fermented *Moringa* leaf sample did not show significant ethanol concentration in the broth whereas, the fermented leaf broth contained about $4.1 \pm 0.09\%$ ethanol content as estimated by the dichromate method. The concentration of ethanol was found to be 0.72 M at a pH of

around 4. For 5% of the inoculum, only 4.0% of ethanol was produced during the fermentation of Agave leaves by *S. cerevisiae* in a similar manner.[17]

Antioxidant Activity

The DPPH assay showed the potential of the fermented *Moringa* leaf broth against free radical scavenging activity. The control sample exhibited only about 40.38 ± 0.21% of antioxidant capacity while the test sample exhibited 67.30 ± 0.34%. In comparison, fermented leaf broth possesses more antioxidant activity than non-fermented broth. The maximum antioxidant activity observed in the aqueous *Moringa* leaf extract was reported to be around 55.1%. Fermentation of plant extracts usually causes abundant hydrolysis of substrates and increases the liberation of antioxidative phenols, peptides, and even flavonoids thus, improving the entire antioxidant property of the fermented leaf broth.

Characterization of the Liposomes

The liposomes encapsulating the fermented *Moringa* leaf broth contained satisfactory liposomal characteristics. The average Zeta Potential of the synthesized liposomes was estimated to be around -27.2 mV with a Zeta Deviation of about 23.3 mV calculated from three different peaks. In addition, the average diameter of the liposomes was found to be 910 nm. The variations obtained in the measurement of Zeta Potential and size of the liposomes may be due to the polydispersity of the sample. In an article, it has been discovered that the Zeta Potentials of liposomes containing *Oregano vulgare* and *Salvia offinalis* extracts were -27.1 ± 4.8 mV and -21.1 ± 0.5 mV respectively and the particle sizes of the formulated liposomes were more than 1000 nm in diameter.[18]

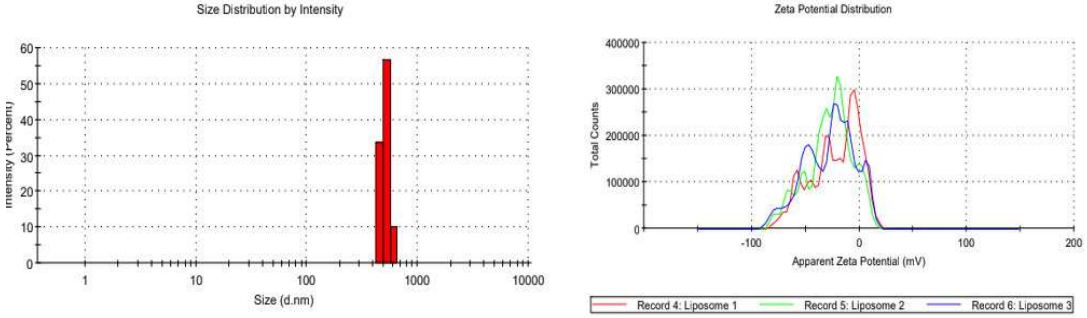

Fig.-2: Particle Size (left) and Zeta Potential (right) of the Liposome

Antibacterial Properties

The fermented leaf broth of *Moringa* has remarkable antibacterial activity than the control aqueous sample. Likewise, the liposomal suspension encapsulating the fermented *Moringa* leaf broth has near similar activity to that of the test samples. The results of all the samples are given in Table-2. Of the three bacteria tested, both the leaf broths and liposomal suspension possess higher inhibitory activity against *Salmonella typhi* than *Klebsiella pneumoniae*. However, the Zone of Inhibition (ZI) for *Bacillus cereus* decreased in the cases of Fermented broth and liposomal suspension. Aqueous extract of *Moringa* leaves at 200 mg/mL concentration exhibited antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella species* with zones of inhibition around 6 ± 0.01 mm, 5 ± 0.11 mm, 8 ± 0.25 and 5 ± 0.14 mm respectively.[15] The presence of peptides with low molecular weight and other phytoconstituents has a well-established antibacterial property. In addition, liposomes help in the delivery of fermented leaf broth (payloads) near the bacterial cells causing decreased bacterial growth around the wells in the petri plates.

Cytotoxicity against MCF-7 Cell Line

An anti-proliferative activity of *Moringa* leaf broth and the liposomal suspension rises in a concentration-dependent manner. Table-3 illustrates the MCF-7 cell line's cytotoxic potential in terms of cell viability % following the addition of of fermented fermented *Moringa* leaf broth and liposomes encapsulating the fermented *Moringa* leaf broth assayed using MTT. To discuss, the IC_{50} concentration *Citrullus vulgaris* extract against the HeLa cell line was estimated to be about 1.020 mg/mL after 24 hours of treatment period.[19] Likewise, the formulated liposomes were capable of targeted delivery of the bioactive fermented

compounds into the cultured MCF-7 cells by fusing with the cell membranes and releasing the anticancer substances.

Table-2: Antibacterial Activities of Non-fermented Broth, Fermented Broth, and Liposomal Suspension Containing Fermented *Moringa* Leaf Broth at 50 µL concentration

Sample	Zone of microbial inhibition for *Bacillus cereus*	Zone of microbial inhibition for *Klebsiella pneumoniae*	Zone of microbial inhibition for *Salmonella typhi*
Non-fermented broth (Control)	16.1 ± 0.4 mm	10.6 ± 0.5 mm	15.3 ± 0.4 mm
Fermented broth (Test)	12.2 ± 0.3 mm	15.4 ± 0.6 mm	22.0 ± 0.8 mm
Liposomal suspension	10.1 ± 0.2 mm	14.4 ± 0.5 mm	16.3 ± 0.7 mm

Table-3: Cell Viability Percentages of Fermented *Moringa* Leaf Broth and Liposomal Suspension at 2, 4, 6, 8, and 10 µL Concentrations against MCF-7 Cell Line

Sample	Cell viability (%) at 2 µL concentration	Cell viability (%) at 4 µL concentration	Cell viability (%) at 6 µL concentration	Cell viability (%) at 8 µL concentration	Cell viability (%) at 10 µL concentration
Fermented *Moringa* leaf broth	83.31%	69.74%	65.12%	56.93%	52.91%
Liposomal suspension	83.79%	79.06%	72.80%	67.89%	64.10%

CONCLUSION

The fermented *Moringa* leaf broth contains various bioactive metabolites relatively higher than that of the simple aqueous broth of *Moringa* leaves. The liberated end products of fermentation by *Saccharomyces cerevisiae* exhibited several biochemical propertiest hat have been quantified and reported. Similarly, the fermented broth also has cytotoxic activities against *Bacillus cereus*, *Klebsiella pneumoniae*, *Salmonella typhi* bacteria, and the MCF-7 cell line. Finally, the liposomes, which contained the fermented *Moringa* leaf broth, and characterized. These liposomes showed significant activities similar to the Test sample. From a future perspective, it can be concluded that fermented plant products may have promising effects on diseases and environments. Likewise, the liposomes could potentially help in the targeted delivery and have positive impacts on the biosystems. Thus, fermented plant extracts can replace the overuse of synthetic products in our day-to-day lives.

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CONFLICT OF INTERESTS

There is no conflict of interest, according to the author.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. S. Khalid, M. Arshad, S. Mahmood, W. Ahmed, F. Siddique, W. Khalid, M. Zarlasht, T. O. Asar and F. A. M. Hassan,*International Journal of Food Properties,* **26**, 1(2023), https://doi.org/10.1080/10942912.2023.2246685
2. H. Shi, B. Su, X. Chen and R. Pian, *PeerJ*, **8**, e10358(2020), https://doi.org/10.7717/peerj.10358
3. H. Q.Chuah, P. L. Tang, N. J. Ang and H. Y. Tan, *Chinese Herbal Medicines,* **13(4)**, 565(2021), https://doi.org/10.1016/j.chmed.2021.09.003
4. A. Starzyńska-Janiszewska, B. Stodolak, C. Fernández-Fernández, B. Mickowska, V.VerardoandA. M. Gómez-Caravaca, *Foods (Basel, Switzerland)*, **11(23)**, 3762(2022), https://doi.org/10.3390/foods11233762
5. P. R. B. Feitosa, T. R. J. Santos, N. C. Gualberto, N. Narain and L. C. L. de Aquino Santana, *Biocatalysis and Agricultural Biotechnology*, **27**, 101709(2020), https://doi.org/10.1016/j.bcab.2020.101709
6. R. Sharma, P. Garg, P. Kumar, S. K. Bhatia and S. Kulshrestha, *Fermentation*, **6(4)**, 106(2020), https://doi.org/10.3390/fermentation6040106
7. K. Jakubczyk, K. Melkis, K. Janda-Milczarek and K. Skonieczna-Żydecka, *Foods (Basel, Switzerland)*, **13(1)**, 102(2023), https://doi.org/10.3390/foods13010102
8. M. W. Ali, M. Z. Ilays, M. T. Saeed, and D. H. Shin, *Journal of Food Science and Technology*, **57(3)**, 1110(2020), https://doi.org/10.1007/s13197-019-04146-1
9. M. Parapouli, A. Vasileiadis, A. S. Afendra and E. Hatziloukas, *AIMS Microbiology*, **6(1)**, 1(2020), https://doi.org/10.3934/microbiol.2020001
10. D. Hwang, H. Charchoghlyan, J. S. Lee, and M. Kim, *International Journal of Food Science & Technology*, **50(9)**, 1988(2015), https://doi.org/10.1111/ijfs.12882
11. F. Alhakmani, S. Kumar and S. A. Khan, *Asian Pacific Journal of Tropical Biomedicine*, **3(8)**, 623(2013), https://doi.org/10.1016/S2221-1691(13)60126-4
12. H. B. Seo,H. J. Kim, O. K. Lee, J. H. Ha, H. Y. Lee andK. H. Jung, *Journal of industrial microbiology & biotechnology*, **36(2)**, 285(2009), https://doi.org/10.1007/s10295-008-0497-4
13. R. J. Pranav, S. S. Kumar, P. R. Rangaraj, K. Sangapillai, and R. Ragunathan, *Food Science And Applied Biotechnology,* **7(1)**, 122(2024), https://doi.org/10.30721/fsab2024.v7.i1.318
14. S. R. G. Moorthy, S. S. Kumar, K. Devandaran, S. Anguchamy, R. Ragunathan and J. Johney, *Journal of Natural Remedies*, **24(5)**, 1091(2024), https://doi.org/10.18311/jnr/2024/36228
15. K. E. Enerijiofi, F. H. Akapo, and J. O. Erhabor, *Bulletin of the National Research Centre*, 45, 179 (2021), https://doi.org/10.1186/s42269-021-00640-9
16. M. A. Chandra, K. Syamsu, L. Ambarsari, N. Fatimah andW. Nurcholis, *Journal of Applied Biology & Biotechnology*, **12(1)**, 111(2024), https://doi.org/10.7324/JABB.2024.149026
17. C. S. Murugan and S. Rajendran, *International Journal of Microbiological Research*, **4(1)**, 23(2013), https://doi/10.5829/idosi.ijmr.2013.4.1.66198
18. T. M. Pereira , C. C. Bonatto and L. P. Silva, *Biosensors*, **13(9)**, 878(2023), https://doi.org/10.3390/bios13090878
19. R. A. Ayo-Lawal, O. Osoniyi, N. R. S. Sibuyi, M. Meyer, and O. Ekpo, *Journal of Dietary Supplements*, **18(2)**, 132(2020), https://doi.org/10.1080/19390211.2020.1731045

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