THE NEW ARMENIAN MEDICAL JOURNAL
Volume18 (2024), Issue 3 p. 106-111

DOI: https://doi.org/10.56936/18290825-3.v18.2024-106

ANTIOXIDANT DRUGS FROM HYDRO-ETHANOLIC FLORAL EXTRACTS OF IMPATIENS BALSAMINA L.: AN IN VITRO ANALYSIS

QAMER S.[1]*, BAKAR I.[2], ALSANOUSI N.[1]

[1] Department of Basic Medical Sciences, College of Medicine, Prince Sattam bin Abdulaziz, University, Al-Kharj, Saudi Arabia

[2] Department of Pathology, Hamdard College of Medicine and Dentistry, Hamdard University, Karachi, Pakistan

Received 16.04.2024; Accepted for printing 04.08.2024

ABSTRACT

Oxidative stress or oxidative cell damage may lead to various systemic or chronic diseases including cancer. Therefore, there is a need to prevent the cells from oxidative stress resulting prevention of disease.

As a consequence of this, our study investigated the anti-oxidant property of hydroethanolic Impatiens balsamina L. flower extracts using various antioxidant assays such as 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay, 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) scavenging assay, catalase assay, hydroxyl radical scavenging activity, and nitric oxide scavenging activity.

The results revealed the potent antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl, 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), catalase, hydrogen peroxide, and nitric oxide scavenging activities. The extract showed efficient inhibitions at 100 µg/ml as 56%, 69%, 67%, 56%, and 59% for 2,2-diphenyl-1-picrylhydrazyl, 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), catalase, hydrogen peroxide, and nitric oxide, respectively.

Overall, hydroethanolic I. balsamina flower extracts had potent antioxidant activity that could be used as a therapeutic agent in the management of oxidative stress.

KEYWORDS: *antioxidant, DPPH, hydroethanolic extract, impatiens* balsamina L.

INTRODUCTION

The medicinal plant is an important source of bioactive components with a wide range of virtually useful properties. Different types of antioxidants synthesized in plants are the main reasons for their application in medicine, aromatherapy, and phototherapy. Antioxidants are significant molecules that inhibit or slow down the process of unstable molecule oxidation resulting in cell damage prevention from free radicals which are short-term molecules, unstable, and extremely reactive owing to their unpaired electrons to bind with nearby molecules to get stability [*Firuzi O et al., 2011; Qazi M, Molvi K, 2018*]. The free radicals can cause cell damage when the molecules get attacked and the free radical's overproduction, as well as inadequate antioxidant production, may lead to oxidative stress responsible for various life-threatening diseases like cancer, diabetes, myocardial infarction, and stroke [*Phaniendra A et al., 2015; Espinosa-Diez C et al., 2015; Pizzino G et al., 2017; Padureanu R et al., 2019; Sharifi-Rad M*

CITE THIS ARTICLE AS:

Qamer S., Bakar I., Alsanousi N. (2024). Antioxidant Drugs From Hydro-Ethanolic Floral Extracts Of Impatiens Balsamina L.: An In Vitro Analysis : A systematic review, The New Armenian Medical Journal, vol.18(2), 106-111; https://doi.org/ 10.56936/18290825-3.v18.2024-106

ADDRESS FOR CORRESPONDENCE:

Dr. Shafqat Qamer,
Department of Basic Medical Sciences, College of Medicine, Prince Sattam bin Abdulaziz, University, Al-Kharj-11942, Saudi Arabia.
Tel.: +966536989328
E-mail: s.ahmed@psau.edu.sa

et al., 2020]. Many endogenous antioxidants such as Catalase, Glutathione peroxidase and Superoxide dismutase can prevent the arisen of oxidative stress but sometimes it fails [*Poprac P et al., 2017*]. Therefore, exogenous antioxidants are extremely needed to prevent these conditions by scavenging free radicals that too safe and more stable as well [*Panova I, Alexander S, 2023*]. Several reports documented, that medicinal plants are rich sources of different antioxidants which are attributed to different phenolic as well as terpene components. Hence, various parts of medicinal plants such as flowers, roots, leaves, and barks are usually used in folk medicines [*Boyles C, Sobeck S, 2020; De Mejia E et al., 2020*]. Therefore, edible flowers from Impatiens balsamina, an ornamental garden plant that is gaining much attention due to their attractive color have been practiced as a folk medicine for several years to treat various diseases by exploring various biological properties such as anti-inflammatory, anti-tumour, anti-microbial, and antioxidant [*Sakunphueak A, Panichayupakaranant P, 2012*]. Hence, the study is mainly focused on exploring the antioxidant properties of hydroethanolic flower extract of I. Balsamina using various parameters.

Materials and methods

Preparation of hydroethanolic flower extract: The hydroethanolic flower extract was prepared by immersing 5*g* of washed fresh flower of I. Balsamina in (70:30) ethanol and water mixtures in a well-closed Erlenmeyer flask. Then, the flask was heated at 25°C until the flowers were detaining their color. Later, the soaked material was filtered using Whatman No 1 filter paper. The filtrate was concentrated by evaporating the ethanol and the obtained crude product was used for further use to analyse the antioxidant properties [*Adigun N et al., 2020*]. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay: The radical scavenging capacity of hydro-ethanolic flower extract of *I. balsamina* was determined using DPPH assay as mentioned earlier [*Su B et al., 2012*]. In brief, 3 *ml* of various concentrations of flower extract were added to the appropriate test tubes followed by the addition of 1 *ml* of methanolic DPPH (0.1 *mM*). This obtained mixture was placed in a dark environment for 30 *mins* and then, the optical density was measured at 517 *nm*. The percentage of radical scavenging activity of hydroethanolic flower extract of I. balsamina was calculated by formula 1:

$$\text{Scavenging effect (\%)} = \frac{OD_B - OD_S}{OD_B} \times 100 \qquad (1)$$

where OD_B is blank optical density, and OD_S is sample optical density.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging assay: The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activity of hydro-ethanolic flower extract of I. balsamina was determined using the method summarized in a previous study [*Pacifico S et al., 2018*]. Before analysis, the ABTS solution was prepared by adding 7 *mM* ABTS in H_2O with 2.45 *mM* potassium persulfate ($K_2S_2O_8$), in the dark at room temperature for 16 *h*. Later, ABTS solution was diluted in 0.1 *M* sodium phosphate buffer (pH 7.4) to get an absorbance value of 0.750 ± 0.025 at 734 *nm*. The various concentrations of flower extract were added in 1 *ml* of ABTS solution and then, the absorbance was measured at 734 *nm* 6 *min* after mixing the solution. The percentage of radical scavenging of hydroethanolic flower extract of I. balsamina was calculated by formula 1.

Catalase assay: To explore the catalase activity of hydro-ethanolic flower extract of I. balsamina was studied using the catalase assay as described before [*Truong V et al., 2023*]. For the assay, the various concentrations of flower extract were added in 1.5 *ml* of phosphate buffer and then, the addition of hydrogen peroxide (60 *mM*). The rate of hydrogen peroxide decomposition was specified by a decrease in absorbance at 240 *nm*. The percentage of radical scavenging of hydroethanolic flower extract was calculated by formula 1.

Hydroxyl radical scavenging activity: The hydro-ethanolic flower extract of I. balsamina scavenging activity on hydroxyl radical was evaluated as mentioned earlier [*Guo T et al., 2011*]. For analysis, different concentrations of hydro-ethanolic flower extract were added in the reaction mixture containing Ferric Chloride or Iron (III) Chloride ($FeCl_3$) - (1 *mM*), 1,10-phenanthroline (1 *mM*), phosphate buffer (0.2 M; pH 7.8) and Hydrogen Peroxide (0.17 M) and incubated for 5 *min* at room tempera-

ture. After that, the reaction mixture was measured at 560 *nm*. The percentage of radical scavenging of hydroethanolic flower extract of I. balsamina was calculated by formula 1.

Nitric oxide scavenging activity: To determine the hydro-ethanolic flower extract of I. balsamina scavenging activity on nitric oxide was measured as mentioned previously [*Alam M et al., 2013*]. In short, various concentrations of hydro-ethanolic flower extract were added to the reaction mixture containing sodium nitroprusside (10 *mM*) in phosphate buffer (0.5 *M*; pH 7.4) and incubated for 60 *min* at 37°C. Then, an equal volume of Griess solution (1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid 1:1 (v/v) was added to the reaction mixture. The OD was read at 540 nm after pink color formation and the percentage of radical scavenging of hydroethanolic flower extract of I. balsamina was calculated by formula 1.

Results

DPPH free radical scavenging assay: The hydro-ethanolic flower extract of I. balsamina radical scavenging capacity was determined using DPPH assay and the obtained result is presented in figure 1. As seen in the figure, the graph was plotted against untreated samples indicating the percentage of inhibitory effect on DPPH free radicals after treatment with varying concentrations such as 10,20,40,60,80 and 100 *µg/ml* of hydroethanolic extract of I. balsamina. The results revealed an increasing percentage of inhibition was observed when increasing concentrations. The maximum inhibition of 56% was noted at 100 *µg/ml* hydro-ethanolic flower extract of I. balsamina.

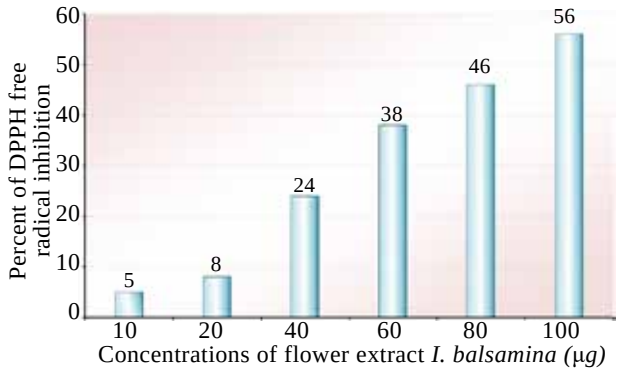

Figure 1. *DPPH free radical scavenging activity of hydroethanolic flower extract of I. balsamina.*

ABTS scavenging assay: The ABTS scavenging activity of the hydro-ethanolic flower extract of I. balsamina evaluated is presented in figure 2. The figure indicated the percentage of hydro-ethanolic flower extract ABTS scavenging activity after treatment with various concentrations (10,20,40,60,80 and 100 *µg/ml*) and also gradual inhibition was observed when the concentration increased. The extract showed maximum scavenging activity as 69% at 100 *µg/ml* of flower extract.

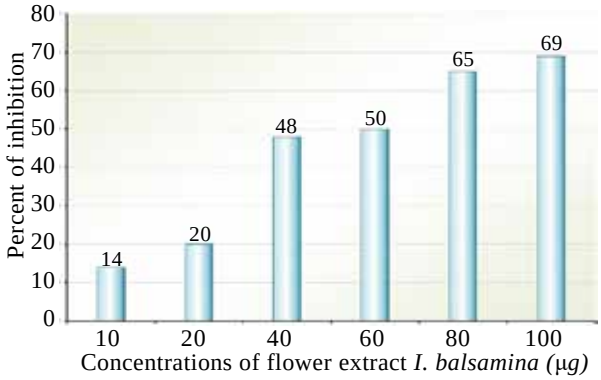

Figure 2. *ABTS scavenging activity of hydroethanolic I. balsamina flower extract.*

Catalase assay: The hydro-ethanolic flower extract catalase activity was explored using the catalase assay and the obtained percentage of catalase activity after treatment is displayed in figure 3. The figure indicates the catalase activity percentage after treatment with various concentrations (10, 20, 40,60, 80, and 100 *µg/ml*) of flower extract, and also, the gradual increase of inhibition was noted while increasing the concentration. The maximum catalase activity was observed as 67% at 100 *µg/ml*.

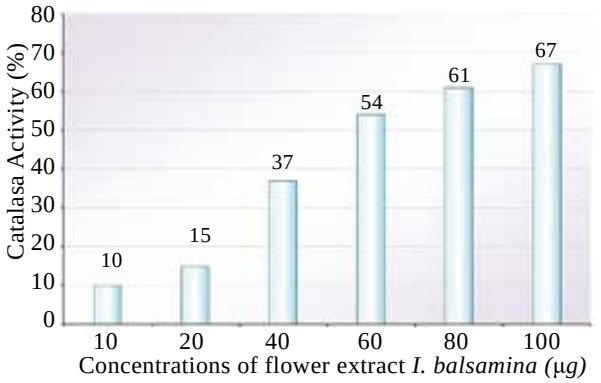

Figure 3. *Hydro-ethanolic flower extract catalase activity*

Hydroxyl radical scavenging activity: The hydro-ethanolic flower extract of I. balsamina scavenging activity on hydroxyl radical evaluated is presented in figure 4: As shown in the figure, the percentage of hydroxyl radical scavenging activity of flower extract various concentrations (10, 20, 40, 60, 80, and 100 *µg/ml*) showed increasing inhibition of hydroxyl radicals and the maximum inhibitory activity was observed as 56% after treatment with 100 *µg/ml*.

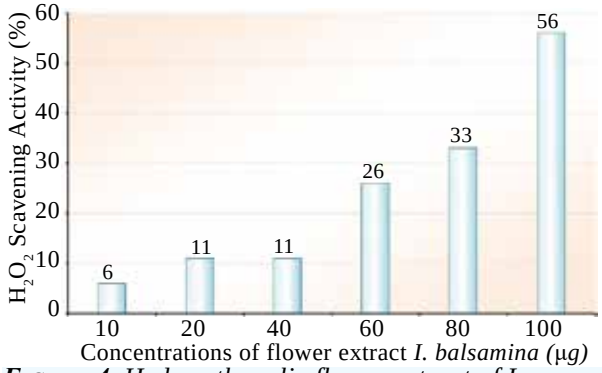

Figure 4. *Hydro-ethanolic flower extract of I. balsamina scavenging activity on hydroxyl radicals.*

Nitric oxide scavenging activity: The hydro-ethanolic I. balsamina flower extract scavenging activity on nitric oxide determined is presented in figure 5. The figure indicated the percentage of nitric oxide scavenging activity after treatment with different concentrations such as 10, 20, 40, 60, 80, and 100 *µg/ml* of flower extract which showed 59/% of inhibition at 100 *µg/ml* of flower extract concentration.

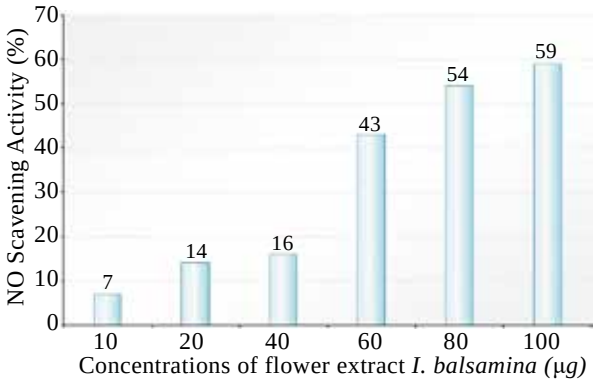

Figure 5. *Hydro-ethanolic I. balsamina flower extract scavenging activity on nitric oxide.*

Discussion

Antioxidants are substances that prevent or slow down the oxidation process resulting in protection from various types of oxidative damage caused by reactive oxygen species that are associated with a variety of diseases including cancers and cardiovascular diseases [*Mohamed S, Khan J, 2013*]. Therefore, antioxidants from natural sources are gaining much attention due to their safety and cost-effectiveness. Hence, our study investigated the antioxidant activity of hydroethanolic I. Balsamina flower extract using various experimental procedures such as DPPH, ABTS, catalase, hydrogen peroxide and nitric oxide scavenging activity revealed potent antioxidant properties. In support of this, a recent study revealed the antioxidant and anti-inflammatory efficiency of lipophilic fractions of Liriope platyphylla seeds using several antioxidant procedures like superoxide anion, DPPH, hydrogen peroxide, ABTS, nitric oxide, lipid peroxidation, hydroxyl radicals scavenging, total antioxidant capacity and reducing antioxidant powers. In addition, this fraction showed elevated cellular antioxidant capacity through reactive oxygen species formation inhibition along with potent inflammatory activity. Altogether, the combined data indicated that lipophilic fractions of Liriope platyphylla seeds may be an effective antioxidant and anti-inflammatory agent [*Truong V et al., 2023*]. Similarly, the antioxidant and phytochemical analysis of C. edulis and P. capensis extracts were evaluated through various parameters like hydroxyl radical, DPPH radical scavenging activity, Iron chelating and ferric reduction, superoxide dismutase, glutathione reductases and catalase revealed the potent antioxidant property in the presence of different types phytochemicals such as flavonoids, phenolics, tocopherols and terpenoids which provide the validation to the extracts used in oxidative stress management [*Muruthi C et al., 2023*]. Similarly, elemicin was isolated from n-hexane seed extract of Myristica fragrans and analysed for their antioxidant property using Lipid peroxidase, catalase, and DPPH assays which showed potent antioxidant properties. In addition, the compound was examined for its antimicrobial activity revealing its potent activity against various clinically relevant pathogens which suggested the promising biological property of Myristica fragrans that can be used for

the development of food preservatives and therapeutic agents [*Al-Qahtani W et al., 2022*]. In vitro, study revealed the Acalypha indica root methanolic extract has antioxidant as well as anti-inflammatory properties. Further, in vivo analysis reduced the edema level after treatment along with a significant reduction in white blood cell, C-reactive protein and platelets. These findings demonstrated the Acalypha indica root methanolic extract has potent antioxidant and anti-inflammatory properties which can be used for the drug development process [*Sahukari R et al., 2021*]. The antioxidant and anti-inflammatory effect of *Schinus terebinthifolius* Raddi leaves ethyl acetate extract was investigated using various anti-oxidant assays and showed potent antioxidant activity by the presence of phytochemicals such as gallic acid or quercetin equivalents, Phenol and flavonoid contents [*da Silva Nascimento M et al., 2023*]. Overall, the plant material and its products have potent antioxidant properties that can be used for the development of therapeutic agents.

Conclusion

The hydroethanolic I. Balsamina flower extract was investigated for its antioxidant properties using different antioxidant assays. The extract showed potent antioxidant activity which was evidenced by analysing various parameters. The result provides scientific evidence of the hydroethanolic I. Balsamina flower extract has antioxidant activity and it can be used for the management of oxidative stress.

Acknowledgment: *The authors are grateful to the Deanship of Scientific Research, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia for its support and encouragement in conducting the research and publishing this report.*

REFERENCES

1. *Adigun NS, Oladiji AT, Ajiboye TO (2020).* Hydro-Ethanolic Fruit Extract of *Capsicum frutescens* Reversed Triton-X-100–Induced Hyperlipidaemia in Rats, Journal of Dietary Supplements. 17(1): 53-66 DOI: 10.1080/19390211.2018.1482982
2. *Alam MN, Bristi NJ, Rafiquzzaman M (2013).* Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharm J. 21: 143-152 DOI: 10.1016/j.jsps.2012.05.002
3. *Al-Qahtani WH, Dinakarkumar Y, Arokiyaraj S, Saravanakumar V, Rajabathar JR, Arjun K, Gayathri PK, Nelson Appaturi J (2022).* Phytochemical and biological activity of *Myristica fragrans*, an ayurvedic medicinal plant in Southern India, and its ingredient analysis. Saudi J Biol Sci. 29(5): 3815-3821 DOI: 10.1016/j.sjbs.2022.02.043
4. *Boyles C, Sobeck, SJS (2020).* Photostability of organic red food dyes. Food Chem. 315: 126249 DOI: 10.1016/j.foodchem.2020.126249
5. *da Silva Nascimento M, Dos Santos PH, de Abreu FF, Shan AYKV, Amaral RG., et al (2023).* Schinus terebinthifolius Raddi (Brazilian pepper) leaves extract: in vitro and in vivo evidence of anti-inflammatory and antioxidant properties. Inflammopharmacology. 31(5): 2505-2519 DOI: 10.1007/s10787-023-01316-8
6. *De Mejia EG, Zhang Q, Penta K, Eroˇglu A, Lila MA (2020).* The colors of health: Chemistry, bioactivity, and market demand for colorful foods and natural food sources of colorants. Annu Rev Food Sci Technol. 11: 145-182 DOI: 10.1146/annurev-food-032519-051729
7. *Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, Lamas S (2015).* Antioxidant responses and cellular adjustments to oxidative stress. Redox Biol. 6: 183-197 DOI: 10.1016/j.redox.2015.07.008
8. *Firuzi O, Miri R, Tavakkoli M, Saso L (2011).* Antioxidant therapy: Current status and future prospects. Curr Med Chem. 18: 3871-3888 DOI: 10.2174/092986711803414368
9. *Guo T, Wei L, Sun J, Hou Cl, Fan L (2011).* Antioxidant activities of extract and fractions from Tuber indicum Cooke & Massee. Food Chem. 127: 1634-1640 DOI: 10.1016/j.foodchem.2011.02.030
10. *Mohamed SA, Khan JA (2013).* Antioxidant capacity of chewing stick miswak Salvadora persica. BMC Complement Altern Med. 40: DOI: 10.1186/1472-6882-13-40
11. *Muruthi CW, Ngugi MP, Runo SM, Mwitari PG (2023). In vitro* antioxidant activities of *Carissa edulis* (Forssk) Vahl) and *Pappea capen-*

sis (Eckyl. & Zeyh) extracts. Heliyon. 9(1): e12965 DOI: 10.1016/j.heliyon.2023.e12965

12. *Pacifico S, Galasso S, Piccolella S, Kretschmer N, Pan S.-P, Nocera P, Lettieri A, Bauer R, Monaco P (2018).* Winter wild fennel leaves as a source of anti-inflammatory and antioxidant polyphenols. Arab J Chem. 11: 513-524 DOI: 10.1016/j.arabjc.2015.06.026

13. *Padureanu R, Albu CV, Mititelu RR, Bacanoiu MV, Docea AO, Calina D, Padureanu V, Olaru G, Sandu RE, Malin RD (2019).* Oxidative Stress and Inflammation Interdependence in Multiple Sclerosis. J Clin Med. 8: 1815 DOI: 10.3390/jcm8111815

14. *Panova IG, Alexander ST (2023).* Endogenous and Exogenous Antioxidants as Agents Preventing the Negative Effects of Contrast Media (Contrast-Induced Nephropathy) Pharmaceuticals. 16(8): 1077 DOI: 10.3390/ph16081077

15. *Phaniendra A, Jestadi DB, Periyasamy L (2015).* Free radicals: Properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 30: 11-26 DOI: 10.1007/s12291-014-0446-0

16. *Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F., et al (2017).* Oxidative stress: Harms and benefits for human health. Oxid Med Cell Longev. 8416763 DOI: 10.1155/2017/8416763

17. *Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M (2017).* Targeting free radicals in oxidative stress-related human diseases. Trends Pharmacol Sci. 38: 592-607 DOI: 10.1016/j.tips.2017.04.005

18. *Qazi MA, Molvi KI (2018).* Free radicals and their management. Am J Pharm Health Res. 6: 1-10 DOI: 10.46624/ajphr.2018.v6.i4.001

19. *Sahukari R, Punabaka J, Bhasha S, Ganjikunta VS, Kondeti Ramudu S., et al (2021).* Phytochemical Profile, Free Radical Scavenging and Anti-Inflammatory Properties of *Acalypha Indica* Root Extract: Evidence from In Vitro and In Vivo Studies. Molecules. 26(20): 6251 DOI: 10.3390/molecules26206251

20. *Sakunphueak A, Panichayupakaranant P (2012).* Comparison of antimicrobial activities of naphthoquinones from Impatiens balsamina. Nat Prod Res. 26(12): 1119-1124 DOI: 10.1080/14786419.2010.551297

21. *Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L., et al (2020).* Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Front. Physiol. 11: 694 DOI: 10.3389/fphys.2020.00694

22. *Su BL, Zeng R, Chen JY, Chen CY, Guo JH, Huang CG (2012).* Antioxidant and antimicrobial properties of various solvent extracts from Impatiens balsamina L. stem. J Food Sci. 77: C614-C619 DOI: 10.1111/j.1750-3841.2012.02709.x

23. *Truong VL, Bae YJ, Rarison RHG, Bang JH, Park SY, Jeong WS (2023).* Anti-Inflammatory and Antioxidant Activities of Lipophilic Fraction from *Liriope platyphylla* Seeds Using Network Pharmacology, Molecular Docking, and In Vitro Experiments. Int J Mol Sci. 24(19): 14958 DOI: 10.3390/ijms241914958

THE NEW ARMENIAN MEDICAL JOURNAL

Volume18 (2024). Issue 3

The Journal is founded by
Yerevan State Medical
University after M. Heratsi.

Rector of YSMU

Armen A. Muradyan

Address for correspondence:

Yerevan State Medical University
2 Koryun Street, Yerevan 0025,
Republic of Armenia

Phones:

(+37410) 582532 YSMU
(+37493 588697 Editor-in-Chief
Fax: (+37410) 582532
E-mail:namj.ysmu@gmail.com, ysmiu@mail.ru
URL:http//www.ysmu.am

Our journal is registered in the databases of Scopus, EBSCO and Thomson Reuters (in the registration process)

Scopus *EBSCO*
Reuters

Copy editor: Tatevik R. Movsisyan

Printed in "LAS Print" LLC
Director: Suren A. Simonyan
Armenia, 0023,Yerevan,
Acharyan St. 44 Bulding,
Phone: (+374 10) 62 76 12,
E-mail: las.print@yahoo.com

THE NEW ARMENIAN MEDICAL JOURNAL
Volume18 (2024). Issue 3

CONTENTS