LE STUDIUM
Loire Valley
Institute for Advanced Studies

LE STUDIUM Multidisciplinary Journal
ISSN 2669-7807

www.lestudium-ias.com

FELLOWSHIP FINAL REPORT

Establishment of Callus Cultures of *Echinacea purpurea* and investigating the Impact of Melatonin on its Secondary Metabolism

Bilal Haider Abbasi[a,b,c], Tehreem Mahmood[b], Arnaud Lanoue[a], Nathalie Giglioli Guivarch[a]

[a] EA2106 Biomolécules et Biotechnologies Végétales, Université de Tours, 37000 Tours, France

[b] Department of Biotechnology, Quaid-i-Azam University, Islamabad 45320, Pakistan

c LE STUDIUM Institute for Advanced Studies, 45000 Orléans, France

REPORT INFO

Fellow: **XXX**
From XXX
Host laboratory in region Centre-Val de Loire: XXX
Host scientist: **XXX**
Period of residence in region Centre-Val de Loire: XXX

Keywords :
Thidiazuron, melatonin, in vitro cultures, HPLC, Callus cultures, E. purpurea.

ABSTRACT

One of the Asteraceae family's most significant therapeutic herbs is Echinacea purpurea (L.) Moench. The plant is extremely rich in anti-inflammatory and antioxidant chemicals, that can be produced effectively and sustainably by in vitro cultures, which are often enhanced by the inclusion of an elicitor in the culture media. In the present investigation, TDZ was effectively utilized to induce E. purpurea callus cultures. Additionally, different melatonin dosages were evaluated for their impact on biomass accumulation, antioxidant capability, and secondary metabolite synthesis. In callus treated with 25µM melatonin, the highest biomass accumulation, total phenolic output, and total flavonoid production were noted. At the same moderate concentration, the best DPPH radical scavenging activity and overall antioxidant capacity were also observed. A positive association was reflected between biomass and these factors. Notable inhibitory effects were seen against pancreatic lipase, alpha-glucosidase, and alpha-amylase after administration with exogenous melatonin, respectively, during the investigation of the potential of callus cultures. These results emphasized the need to look into the existing strategy in more detail in order to identify novel approaches to treating diabetes and obesity. In HPLC analysis, maximum amounts of metabolites resulted at concentrations of 25–50 µM, except amino acids that were associated with the lowest melatonin concentration. Our research showed that TDZ can be used for efficient callus induction of E. purpurea, and elicitation with melatonin may be a useful tactic for boosting biomass, phenolic compounds, flavonoids, and antioxidant capacity as well as numerous enzyme inhibitory effects.

1- Introduction

Echinacea purpurea, the renowned purple coneflower, is a perennial herb that exhibits potent medicinal properties. From common cold to deadly cancer, the plant offers numerous benefits to prevent these ailments. The phytochemical composition of plant is highly complex and the substances from the roots and herbs, such as, ketoalkenes, caffeic acid derivatives, polysaccharides, and glycoproteins, are responsible for noted immunostimulatory and anti-inflammatory activities [1]. Although, wild plants are subjected to seasonal constraints, the *in vitro* grown plants can offer a year-round production of essential metabolites and this accumulation in plant tissue culture can be augmented by different techniques among which, elicitation is one of the most employed [2]. Exogenous melatonin administration has proven to boost photosynthetic carbon uptake, enhance biomass production, encourage the

production of cold-responsive genes, enhance plant disease resistance, promote tolerance against salinity, and reduce redox imbalance [3]. The study aims to establish *in vitro* callus cultures of purple coneflower, along with an objective of enhancing its secondary metabolism by employing melatonin as an elicitor.

2- Experimental details

Seeds of purple coneflower were collected from University De Tours, France. Following the surface sterilization with mercuric chloride and ethanol, they were inoculated over the basal media, and transferred to a growth chamber which is maintained at optimal conditions.

Once the *in vitro* plantlets were grown, small pieces of leaves and stems were employed as explant for callus induction. Three different media containing, each containing a distinct hormone, including TDZ, BAP, and NAA, were checked for their ability to induce callus production. Subsequently, several melatonin doses were chosen to elicit the metabolism of optimized callus cultures. Different concentrations of melatonin (0, 1, 5, 10, 25, and 50 μM) were supplemented to the media containing 3TDZ. Optimized media containing particular amounts of elicitor were employed for the callus induction. The resulting callus was weighed, dried, and ground to fine powder to access the impact of specific concentration of each elicitor on total phenolic and flavonoid content, antioxidant potential, enzyme-inhibitory affects, and metabolic profile of the plant.

The amount of TPC was calculated by mixing the Folin-Ciocalteu reagent with the samples and sodium carbonate [4]. The absorbance at a wavelength of 630 nm was checked. Gallic acid was employed as a standard and the formula shown below was employed to determine total phenolic production. For evaluating the total flavonoid content, Aluminum trichloride solution and potassium acetate solution were added to samples along with distilled water [5]. The wavelength of 415 nm was used to determine the absorbance. As a standard, Quercetin was employed, and the formula given below was employed to evaluate the total flavonoid production.

The elicited callus cultures were subjected to DPPH assay to determine their free radical scavenging activity (FRSA). DPPH reagent was added to samples and after an hour's incubation in dark, absorbance was measured at 517 nm, while keeping ascorbic acid as a control [6]. Total Antioxidant Capacity (TAC) was evaluated by phosphomolybdenum assay, in which a reagent, including ammonium molybdate, sulfuric acid, and sodium phosphate, was added to samples. Using ascorbic acid as a control, absorbance was checked at 695 nm [7].

Callus cultures were evaluated for their ability to inhibit α-amylase, α-glucosidase, and pancreatic lipase *in vitro* [8] [9].

A solution of α-Amylase was made using a phosphate buffer. A small quantity of the sample was combined with α-amylase and a starch solution to start the test, and it was then incubated for intervals of time at 30°C. The reaction ceased by DNSA reagent after a predetermined amount of time, and the mixture's absorbance (540 nm) was calculated. Acarbose served as a positive control to enable results comparison. α-glucosidase solution was prepared in sodium phosphate buffer. A ten-minute incubation was allowed after mixing the solution with samples. ρ-NPG substrate solution was added and placed in dark. Yellow-colored ρ-nitrophenol was finally determined for its absorbance at 405 nm. Acarbose was utilized as the positive control agent in this assay. A lipase solution was made using 20 mM Tris-HCl. The solution was mixed with samples and incubated at 30°C. Next, 4-MUO substrate solution was added, and following incubation for short time, lipase produced 4-methylumbelliferone fluorescence at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

To investigate the metabolic profile of callus cultures, HPLC analysis was used. The separations were carried out using a C18 column and an acetonitrile and water elution gradient. 10% B (16–20 min), 100% B (12–16

min), and 10–60% B (0–12 min) made up the elution profile. For sample analysis, a 1µL injection volume was used in conjunction with the Fast Data-dependent Acquisition Mode (fDDA). To compare metabolites abundance between samples, peak areas were divided by the ratio dry weight of plant/ extract weight. Before LC/MS analysis, standard solutions were made by sonicating plant materials in methanol with formic acid and resuspension in MeOH.

Every experiment was performed thrice, in triplicates. Average values of each experiment were calculated, and standard error was determined using Microsoft Excel program.

3- Results and discussion

One of the most important factors influencing the induction, growth, and synthesis of vital plant bioactive components in culture media is the amount of PGRs [10]. After the seeds were inoculated, they took about two months to grow explants, which were then used to establish callus. After one month of inoculation on medium with various PGR treatments (NAA, BAP, and TDZ), induction of callus from leaf and stem explants was evaluated.

There was a small amount of callus induced in media containing BAP, and adventitious rooting was observed in media containing NAA. On the other hand, biomass accumulation and successful callus induction were the outcomes of using TDZ. This might be as a result of the identification of the induction of adventitious root formation as one of NAA's characteristics [11]. Furthermore, TDZ is more effective at plant regeneration than BAP, according to a number of studies [12]. The discovery that TDZ may substitute for both auxin and cytokinin requirements is consistent with earlier research utilizing a variety of regeneration systems, which shown that using TDZ as the only growth regulator resulted in a high frequency of callogenesis [13]. The analysis of current study showed that, when utilizing stem explants, MS media incubated with 3 mg/L TDZ produced the maximum callus induction frequency.

In comparison to the control, all melatonin doses increased biomass output. The highest biomass was collected at a moderate dosage of melatonin among the elicited cultures, demonstrating a good callogenic response. Up to a certain point, as the melatonin content increased, biomass output increased gradually. After that concentration, melatonin levels rose, and biomass started to decrease. The biomass synthesis was inhibited, and the callus darkened in the media with the highest melatonin levels, which may have been caused by either excessive production of certain phytochemicals or cell death. The findings are consistent with previous research showing that biomass production decreased when melatonin content was raised to a certain point [11]. This may be the result of stress brought on by elevated concentrations and formation of ROS, which prevent cells from multiplying [14].

Out of all the melatonin treatments, 25µM melatonin showed the highest TPC, while callus grown on MS medium with the highest melatonin concentration showed the lowest TPC. It is well known that the phenylpropanoid pathway is activated in response to adverse environmental conditions, including metals, salt, drought, UV radiation, and extreme temperatures, which results in the accumulation of different phenolic chemicals [15][16]. Because of the extreme stress conditions in the wild as contrasted to *in vitro* environments, callus cultures often have lower levels of phenolic and flavonoid chemicals than wild plants [17][18].

Plants rely on flavonoids for a multitude of functions, such as protection against UV radiation damage, defense against phytopathogens, legume nodulation, male fertility, visual signaling, and regulation of auxin transport [19]. The formation of flavonoids followed the same pattern as that of phenolic components [20]. Though it is usually recognized that mostly phenolic content is greater than flavonoid content, we found an opposite trend in our research. It might be due to the polarity of the extracting solvents or the fact that nonphenolic chemicals do react with the FC reagent [21][22].

An intricate balance between the production and elimination of reactive oxygen species (ROS) is necessary for good cellular homeostasis. Significant levels of ROS result in oxidative damage when this equilibrium is upset by different environmental stressors [23]. Plants have an internal defence system to mitigate the harmful effects of oxidative stress. Many secondary metabolites are produced by plants to shield their cells from oxidative damage [24].

A free radical used in the DPPH assay is typically paramagnetic and becomes a stable diamagnetic molecule after absorbing an electron or a free radical species. As a result, it changes from purple to yellow in color. The callus that was developed with a moderate dose of melatonin had the highest DPPH-FRSA, However, the potential for antioxidants was reduced at the highest melatonin concentration. Our results concur with previous studies showing that antioxidant potential increased quickly following control and that, after a certain threshold was reached, exposure to the highest concentrations of melatonin decreased the antioxidant activity [25][26].

The phosphomolybdate technique has been widely used to evaluate the presence of extracts with antioxidant potential, by which Mo (VI) is reduced to Mo (V), forming a green complex [27]. The use of the phosphomolybdate assay to assess the antioxidant potential of *E. purpurea* callus cultures has not yet been documented in any study. Except for extremely high melatonin dose, all of the melatonin doses used in our current research increased TAC from the control. The existence of more secondary metabolites that can convert Mo (VI) to Mo(V) may be the cause of the increased TAC readings.

Additionally, a biomass-dependent findings and direct correlation between the accumulation of flavonoids, phenolics, FRSA, and total antioxidant capacity was shown which agrees with the prior reports [28][29].

To lower blood glucose levels with the aim of preventing diabetes, one therapeutic technique is to inhibit the enzymes alpha-glucosidase and alpha-amylase. Phenolic acids, among other phytochemicals, from the wild E. purpurea plant have the potential to improve diabetes glucose control by blocking the activity of α-amylase and α-glucosidase [30]. The current investigation found that the maximum inhibition against the amylase enzyme was demonstrated by callus cultures treated with the highest and second highest elicitor doses. Just two doses inhibited the enzyme, demonstrating a positive reaction. Every other concentration displayed a negative value, indicating the absence of inhibition. It could be the consequence of an alteration in conformation caused by materials binding to the enzyme [31].

The metabolism of carbohydrates also depends on the enzyme α-glucosidase. Callus cultures were used to access its inhibition, and the results showed that the largest percentage of α-glucosidase inhibition was allowed with a moderate level of melatonin. Our results are in line with another study where melatonin treatment showed efficacy in enhancing the antidiabetic effect of *Lepidium sativum* callus extracts [32].

Research on pancreatic lipase inhibition as a potential anti-obesity medication approach is getting advanced [33]. To the best of our knowledge, this work is the only one looking at the potential anti-obesity benefits of *E. purpurea* callus cultures using *in vitro* tests. According to our results, the callus treated with 10 μM melatonin exhibited the highest and most increased percentage of lipase inhibition when compared to the non-elicited culture. Additionally, the maximum elicitor dose exhibited significant efficacy against pancreatic lipase. Many both *in vivo* and *in vitro* research have indicated that melatonin can improve the anti-obesity capabilities [34]. The enzyme was somewhat inhibited by all other concentrations as well, but the percentage of inhibition was lower than that of the control. The contradictory outcomes could be explained by melatonin's dual properties, which function as both pro- and antioxidants and may affect how it interacts with lipase [35].

HPLC-based tests were conducted to examine the effects of varying melatonin dosages on the synthesis of 14 phytochemicals and 5 amino acids in *Echinacea purpurea* callus cultures. Several molecules, including chicoric acid and quercetin 3-O-rutinoside, demonstrated higher production at 50 μM melatonin, while neochlorogenic acid and quercetin 3-O-(6-O-malonyl-glucoside) showed maximal production at 25 μM melatonin. This points to a possible biphasic response, implying that compound synthesis is at its best within a particular melatonin range. Thus, melatonin can act as a stimulant to improve the synthesis of phytochemicals with high economic value.

The production of amino acids in callus cultures of *Echinacea purpurea*, however, showed a distinct pattern. Cultures treated with the lowest melatonin dose (1 μM) exhibited the highest levels of glucogenic amino acids; increasing melatonin concentrations resulted in a drop in amino acid output. Previous studies have suggested that this change may be related to melatonin's effect on the gluconeogenesis pathway. The potential uses of melatonin in the synthesis of chemicals are supported by these subtle insights into phytochemical and amino acid biosynthesis, which encourage more molecular research in areas like natural product discovery, plant biotechnology, and medicine.

4- Conclusion

In this work, *E. purpurea* callus was successfully produced, and the effects of varying melatonin doses were evaluated with respect to biomass accumulation, secondary metabolism, antioxidant capacity, and the potential of callus cultures to block certain enzymes. When melatonin was used as an elicitor, the callus cultures produced more biomass and had higher secondary metabolism. Surprisingly, phenolics, flavonoids, and antioxidant levels rose in response to melatonin as an elicitor. Also, the induced cultures revealed a significant concentration of essential pharmaceutical components using HPLC studies. Additionally, the present investigation demonstrated a significant link between antioxidant potential, phenolic and flavonoid synthesis, and biomass accumulation. It was also shown that a certain range of melatonin treatments had inhibitory potential against enzymes linked to diabetes and obesity. Together, these results highlight how melatonin may help *E. purpurea* callus cultures produce biomass and phytochemicals more sustainably and effectively. The study also highlights the necessity of additional investigation at pilot and commercial scales to confirm and magnify promising outcomes.

5- Perspectives of future collaborations with the host laboratory

A smooth continuation of this collaboration is planned, building on the ongoing work with Prof. Nathalie Giglioli Guivarch, envisioning future breakthroughs in comprehending the features of biotechnological resources.

6- Articles published in the framework of the fellowship

Khan, T., Javed, M.U., Mahmood, T., Khan, B., Khan, T., Ullah, M.A., Khurshid, R., Zaman, G., Hano, C., Giglioli-Guivarc'h, N. and Abbasi, B.H., 2024. Enhancement in the production of phenolic compounds from Fagonia indica callus cultures via Fusarium oxysporum triggered elicitation. In Vitro Cellular & Developmental Biology-Plant, pp.1-12.

DOI: 10.1007/s11627-023-10358-0

7- Acknowledgements

Bilal Haider Abbasi performed the experiments with the assistance of Tehreem Mahmood. HPLC analysis and enzymatic inhibition assays were performed by Arnaud Lanouea. Nathalie Giglioli-Guivarc'h and Bilal Haider Abbasi conceived the idea, facilitated and supervised the research.

8- References

[1] G. Megha, D. Sharma, A. Sharma, V. Kumari, O. P. Goshain, and others, "A review on purple cone flower (Echinacea purpurea L. Moench).," *J. Pharm. Res.*, vol. 5, no. 8, pp. 4076–

4081, 2012.

[2] S. Karuppusamy, “A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures,” *J. Med. Plants Res.*, vol. 3, no. 13, pp. 1222–1239, 2009.

[3] S. Samanta, A. Singh, A. Banerjee, and A. Roychoudhury, “Exogenous supplementation of melatonin alters representative organic acids and enzymes of respiratory cycle as well as sugar metabolism during arsenic stress in two contrasting indica rice cultivars,” *J. Biotechnol.*, vol. 324, pp. 220–232, 2020.

[4] M. Ali, B. H. Abbasi, and Ihsan-ul-haq, “Production of commercially important secondary metabolites and antioxidant activity in cell suspension cultures of Artemisia absinthium L.,” *Ind. Crops Prod.*, vol. 49, pp. 400–406, 2013, doi: 10.1016/j.indcrop.2013.05.033.

[5] M. Shahriar, S. Akhter, M. Hossain, A. Haque, and M. Bhuiyan, “Evaluation of in vitro antioxidant activity of bark extracts of Terminalia arjuna,” *J. Med. plant Res.*, vol. 6, pp. 5286–5298, Oct. 2012, doi: 10.5897/JMPR12.580.

[6] T. Khan, M. A. Ullah, L. Garros, C. Hano, and B. H. Abbasi, “Synergistic effects of melatonin and distinct spectral lights for enhanced production of anti-cancerous compounds in callus cultures of Fagonia indica,” *J. Photochem. Photobiol. B Biol.*, vol. 190, pp. 163–171, 2019, doi: 10.1016/j.jphotobiol.2018.10.010.

[7] M. Zia, H. Zafar, F. Z. Gul, B. H. Abbasi, Z. F. Rizvi, and A. Mannan, “Synergistic influence of CuO nanoparticles and spectral lights transforms biomass, antioxidative response, and antioxidants in Brassica nigra,” *Plant Cell, Tissue Organ Cult.*, vol. 145, pp. 261–274, 2021.

[8] M. N. Wickramaratne, J. C. Punchihewa, and D. B. M. Wickramaratne, “In-vitro alpha amylase inhibitory activity of the leaf extracts of Adenanthera pavonina,” *BMC Complement. Altern. Med.*, vol. 16, pp. 1–5, 2016.

[9] C. Lankatillake *et al.*, “Screening natural product extracts for potential enzyme inhibitors: Protocols, and the standardisation of the usage of blanks in $α$-amylase, $α$-glucosidase and lipase assays,” *Plant Methods*, vol. 17, no. 1, pp. 1–19, 2021.

[10] R. Akula and G. A. Ravishankar, “Influence of abiotic stress signals on secondary metabolites in plants,” *Plant Signal. \& Behav.*, vol. 6, no. 11, pp. 1720–1731, 2011.

[11] H. Fazal, B. H. Abbasi, and N. Ahmad, “Optimization of adventitious root culture for production of biomass and secondary metabolites in Prunella vulgaris L.,” *Appl. Biochem. Biotechnol.*, vol. 174, pp. 2086–2095, 2014.

[12] T. Lazić and \DJur\djina Ružić, “Organogenesis in vitro from the leaf of blackberry cv Čačanska bestrna,” *Genetika*, vol. 39, no. 1, pp. 69–78, 2007.

[13] B. N. S. Murthy, S. J. Murch, and P. K. Saxena, “Thidiazuron: a potent regulator of in vitro plant morphogenesis,” *Vitr. Cell. Dev. Biol.*, vol. 34, pp. 267–275, 1998.

[14] H. Fazal, B. H. Abbasi, N. Ahmad, M. Ali, and S. Ali, “Sucrose induced osmotic stress and photoperiod regimes enhanced the biomass and production of antioxidant secondary metabolites in shake-flask suspension cultures of Prunella vulgaris L.,” *Plant Cell. Tissue Organ Cult.*, vol. 124, no. 3, pp. 573–581, 2016, doi: 10.1007/s11240-015-0915-z.

[15] I. Linić, D. Šamec, J. Grúz, V. Vujčić Bok, M. Strnad, and B. Salopek-Sondi, “Involvement of phenolic acids in short-term adaptation to salinity stress is species-specific among Brassicaceae,” *Plants*, vol. 8, no. 6, p.

155, 2019.

[16] A. Sharma, B. Shahzad, A. Rehman, R. Bhardwaj, M. Landi, and B. Zheng, "Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress," *Molecules*, vol. 24, no. 13, p. 2452, 2019.

[17] G. Schmeda-Hirschmann, M. Jordan, A. Gerth, and D. Wilken, "Secondary metabolite content in rhizomes, callus cultures and in vitro regenerated plantlets of Solidago chilensis," *Zeitschrift für Naturforsch. C*, vol. 60, no. 1–2, pp. 5–10, 2005.

[18] M. Stanišić *et al.*, "Clonal fidelity of Iris sibirica plants regenerated by somatic embryogenesis and organogenesis in leaf-base culture - RAPD and flow cytometer analyses," *South African J. Bot.*, vol. 96, pp. 42–52, 2015, doi: 10.1016/j.sajb.2014.10.014.

[19] X. Wang *et al.*, "Flavonoids and saponins: What have we got or missed?," *Phytomedicine*, p. 154580, 2022.

[20] S. Cheng, X. U. Feng, L. I. Linling, H. Cheng, and W. Zhang, "Seasonal pattern of flavonoid content and related enzyme activities in leaves of Ginkgo biloba L.," *Not. Bot. Horti Agrobot. Cluj-Napoca*, vol. 40, no. 1, pp. 98–106, 2012.

[21] J. D. Everette, Q. M. Bryant, A. M. Green, Y. A. Abbey, G. W. Wangila, and R. B. Walker, "Thorough study of reactivity of various compound classes toward the Folin- Ciocalteu reagent," *J. Agric. Food Chem.*, vol. 58, no. 14, pp. 8139–8144, 2010.

[22] N. F. Jaafar, M. E. Ramli, and R. M. Salleh, "Optimum extraction condition of Clitorea ternatea flower on antioxidant activities, total phenolic, total flavonoid and total anthocyanin contents," *Trop. life Sci. Res.*, vol. 31, no. 2, p. 1, 2020.

[23] K. Das and A. Roychoudhury, "Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants," *Front. Environ. Sci.*, vol. 2, p. 53, 2014.

[24] R. Mittler, S. I. Zandalinas, Y. Fichman, and F. Van Breusegem, "Reactive oxygen species signalling in plant stress responses," *Nat. Rev. Mol. Cell Biol.*, vol. 23, no. 10, pp. 663–679, 2022.

[25] J. Hernández-Ruiz and M. B. Arnao, "Relationship of melatonin and salicylic acid in biotic/abiotic plant stress responses," *Agronomy*, vol. 8, no. 4, p. 33, 2018.

[26] V. N. Sarropoulou, I. N. Therios, and K. N. Dimassi-Theriou, "Melatonin promotes adventitious root regeneration in in vitro shoot tip explants of the commercial sweet cherry rootstocks CAB-6P (Prunus cerasus L.), Gisela 6 (P. cerasus$\times$ P. canescens), and MxM 60 (P. avium$\times$ P. mahaleb).," *J. Pineal Res.*, vol. 52, no. 1, pp. 38–46, 2012.

[27] P. Prieto, M. Pineda, and M. Aguilar, "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E," *Anal. Biochem.*, vol. 269, no. 2, pp. 337–341, 1999.

[28] T. Khan, T. Khan, C. Hano, and B. H. Abbasi, "Effects of chitosan and salicylic acid on the production of pharmacologically attractive secondary metabolites in callus cultures of Fagonia indica," *Ind. Crops Prod.*, vol. 129, no. December 2018, pp. 525–535, 2019, doi: 10.1016/j.indcrop.2018.12.048.

[29] J. M. Canadanovic-Brunet, S. M. Djilas, G. S. Cetkovic, and V. T. Tumbas, "Free-radical scavenging activity of wormwood (Artemisia absinthium L) extracts," *J. Sci. Food Agric.*, vol. 85, no. 2, pp. 265–272, 2005.

[30] S.-Y. Chiou, J.-M. Sung, P.-W. Huang, and S.-D. Lin, “Antioxidant, antidiabetic, and antihypertensive properties of Echinacea purpurea flower extract and caffeic acid derivatives using in vitro models,” *J. Med. Food*, vol. 20, no. 2, pp. 171–179, 2017.

[31] C. Mayasankaravalli *et al.*, “Profiling the phyto-constituents of Punica granatum fruits peel extract and accessing its in-vitro antioxidant, anti-diabetic, anti-obesity, and angiotensin-converting enzyme inhibitory properties,” *Saudi J. Biol. Sci.*, vol. 27, no. 12, pp. 3228–3234, 2020.

[32] M. A. Ullah, D. Tungmunnithum, L. Garros, S. Drouet, C. Hano, and B. H. Abbasi, “Effect of ultraviolet-C radiation and melatonin stress on biosynthesis of antioxidant and antidiabetic metabolites produced in in vitro callus cultures of Lepidium sativum L.,” *Int. J. Mol. Sci.*, vol. 20, no. 7, p. 1787, 2019.

[33] N. A. Lunagariya, N. K. Patel, S. C. Jagtap, and K. K. Bhutani, “Inhibitors of pancreatic lipase: state of the art and clinical perspectives,” *EXCLI J.*, vol. 13, p. 897, 2014.

[34] A. A Al-Joraisi and O. Y AL-Abbasy, “Separation of Lipase from Karnel of Pistacia khinjuke and Determination of it’s Affinity Toward some Inhibitors in Mice In vivo and In vitro,” *Rafidain J. Sci.*, vol. 24, no. 6, pp. 54–74, 2013.

[35] H.-M. Zhang and Y. Zhang, “Melatonin: a well-documented antioxidant with conditional pro-oxidant actions,” *J. Pineal Res.*, vol. 57, no. 2, pp. 131–146, 2014.

[36] T. Khan *et al.*, “Enhancement in the production of phenolic compounds from Fagonia indica callus cultures via Fusarium oxysporum triggered elicitation,” *Vitr. Cell. Dev. Biol. - Plant*, no. 0123456789, 2023, doi: 10.1007/s11627-023-10358-0.