CHEMICAL ENGINEERING TRANSACTIONS
VOL. 100, 2023
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Sauro Pierucci, Flavio Manenti

ISBN 979-12-81206-03-8; **ISSN** 2283-9216
DOI: 10.3303/CET23100050

Comparison of the Release Kinetics of a Natural Drug-Active Extract from Mangifera Indica Leaves Impregnated into Thermoplastic Polyurethane and Polylactic Acid by Supercritical Fluids

Lidia Verano-Naranjo*, Antonio Rincón, Cristina Cejudo-Bastante, Lourdes Casas, Casimiro Mantell, Enrique Martínez de la Ossa

Chemical Engineering and Food Technology Department, Wine and Agrifood Research Institute (IVAGRO), University of Cadiz, Avda. República Saharaui, s/n, 11510, Puerto Real, Cádiz, Spain
lidia.verano@uca.es

The use of pharmacoactive polymeric devices has experienced significant growth in recent years due to their ability to increase the efficacy of treatment and acceptance by the body. Among the natural active principles, those recovered from waste from other industries stand out. In addition to participating in the circular economy, some of these by-products have very interesting properties to be used in biomedicine. However, the challenge in this field is to introduce these active principles into polymers in the most efficient and least polluting way possible. In this sense, one of the techniques with the greatest impact is the use of supercritical fluids. In this work, the supercritical impregnation of two biomedical polymers was studied comparatively: polylactic acid (PLA) and thermoplastic polyurethane (TPU). The impregnated active ingredient was *Mangifera indica* leaf extract (MLE), which has demonstrated antioxidant, antimicrobial, and anti-inflammatory capacities, and proangiogenic, antiproliferative, and anti-apoptotic effects on colony-forming endothelial cells.
The results obtained expose the potential of the polymers studied in the field of biomedicine due to the acquired bioactivity of both polymers after the process, which was verified by measuring the antioxidant capacity. Better loads have been obtained in TPU compared to PLA. Both polymers exhibit diffusion-based release kinetics, in which a rapid release is observed in the early moments followed by a much slower release. However, PLA seems to release a greater proportion of its impregnated extract than TPU in less time, which is interesting to determine the different applications that each polymer can have.

1. Introduction

The use of medicinal plants dates to the origins of the first human civilizations when different combinations were used to obtain health benefits, being the origin of current medicines (Kumar et al., 2022). In recent years there has been an exponential increase in the demand for natural products with applications in the field of medicine.
Mango, *Mangifera indica*, is considered one of the most important tropical fruits and one of the world's most consumed fruits (Ferreira et al., 2019). Only the pulp is consumed from the mango while the leaves, seeds, skin, and stems are discarded, representing between 35 to 60 % of the total biomass of this fruit crop (Pratelli et al., 2022). Processing these by-products as raw materials in other industries favors the circular economy and allows obtaining substances with high added value (Fernández-Ponce et al., 2013).
Mango extracts rich in polyphenols, specially mangiferin, have shown potential in different medical fields, presenting anti-inflammatory (Saha et al., 2016), anti-allergenic (Rivera et al., 2006), antioxidant (Du et al., 2018) or anticancer (Mei et al., 2012) properties among others. A recent study evidences the pro-angiogenic and anti-apoptotic effect of mango leaf extract over endothelial colony-forming cells (Sánchez-Gomar et al, 2022), pointing this extract as a good candidate for its addition into biomedical devices for the tissular engineer.
Numerous polymers can be used in the biomedical field, including polylactic acid (PLA) and thermoplastic polyurethane (TPU). PLA is a leading material for biomedical applications, it is widely used for scaffolds, blood

Paper Received: 11 February 2023; Revised: 28 March 2023; Accepted: 31 May 2023
Please cite this article as: Verano-Naranjo L., Rincon A., Cejudo-Bastante C., Casas L., Mantell C., Martinez De La Ossa E., 2023, Comparison of the Release Kinetics of a Natural Drug-active Extract from Mangifera Indica Leaves Impregnated Into Thermoplastic Polyurethane and Polylactic Acid by Supercritical Fluids, Chemical Engineering Transactions, 100, 295-300 DOI:10.3303/CET23100050

vessels, organs, and skin regeneration (Liu et al., 2020). On the other hand, TPU also plays a vital role in the development of vascular and other medical implantable devices (M'Bengue et al., 2023).
Regarding the addition of active substances in polymers, new impregnation techniques, more effective and ecological than the traditional ones, have been developed in recent years. Among these techniques, the supercritical solvent impregnation (SSI) uses solvents at high pressure, such as supercritical CO_2 alone or in mixtures with cosolvents like ethanol mainly (Zalepugin et al., 2020). Supercritical CO_2 can dissolve the active substances and transport them into the polymeric matrices, escaping when it returns to its gaseous form and while these substances remain trapped. Thus, the use of supercritical impregnation in biomedicine is an essential tool since it solves two limiting problems in using organic solvents. Firstly, it allows greater penetration of the compound of interest into the polymer, which translates into a higher loading. Secondly, the use of organic solvents requires further elimination by high temperatures that can damage the polymeric devices. However, supercritical CO_2 impregnation does not need further treatments, so the polymers are ready to use after processing (Liparoti et al., 2022).
The objective of this work is to study the effect of pressure in the impregnation process of a mango leaf extract (MLE) with high antioxidant power into PLA and TPU, determining the extract loading and the release of the impregnated MLE into a saline medium, studying the evolution of the antioxidant capacity.

2. Materials and methods

2.1 Raw materials and extract

For their functionalization, it was used filaments of polylactic acid (PLA), provided by bq (Madrid, Spain), and filaments of thermoplastics polyurethane (TPU), provided by Greeetech (Shenzhen, China).
The mango leaves extract (MLE) was achieved through Enhance Solvent Extraction (ESE) in batch mode in a supercritical extraction equipment provided by Thar Technologies (Pittsburgh, USA) described in previous work (Verano-Naranjo et al., 2021). About 443 g of previously dried and crushed *Mangifera indica* leaves were introduced in a 1000 mL vessel with 600 mL of ethanol. Then the vessel was heated up to 80 ºC and CO_2 was pumped until reaching 200 bar pressure. After 24 hours in the same conditions, the system was depressurized and cooled and the MLE was obtained.

2.2 Antioxidant capacity of the extract

The antioxidant capacity of the MLE was determined by the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method. For this determination, 7 µL of the extract at different concentrations were mixed with 293 µL of $6 \cdot 10^{-5}$ M DPPH ethanolic solution. The absorbance at 515 nm was measured after 2 hours, when the reaction gets a stationary state, in a Synergy HTX Multi-Mode Reader by BioTek Instruments (Vermont, USA). The same reaction using ethanol instead the extract serves as control of the reaction. The percentage of oxidative inhibition was calculated using equation 1:

$$\%\ oxidation\ inhibition = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \quad (1)$$

The antioxidant activity index was calculated by equation 2, in which IC_{50} is the concentration of MLE necessary for an oxidation inhibition of 50 % and $[DPPH]_r$ is the final concentration of the free radical in the reaction medium.

$$AAI = \frac{[DPPH]_r}{IC_{50}} \quad (2)$$

2.3 Impregnation of the polymers

The SSI process was carried out in similar equipment as the extraction one that operates with a 100-mL vessel. For this purpose, 3 mL of MLE were introduced at the bottom of the vessel together with 7 cm pieces of polymer filaments placed on a metal basket. The system was heated up to 35 ºC and CO_2 was pumped until the set pressure (100, 250, or 400 bar). After 1 hour of treatment, the system was depressurized and cooled and the impregnated polymers were taken. The experiments were carried out in duplicate for both polymers under study.

2.4 Estimation of the amount of extract impregnated

To determine the amount of MLE impregnated, a certain amount of impregnated polymer was dissolved using 4 mL of organic solvent —tetrahydrofuran (THF) for TPU and dichloromethane (DCM) for PLA—. The quantification of the extract contained in the sample was calculated based on the calibration lines previously

made at an adequate wavelength (equations 3 and 4). Measurements were carried out in a UV-Vis spectrophotometer model UVmini-1240 by Shimadzu Corporation (Kioto, Japan).

MLE in DMC (10-300 mg/L): $\text{Abs}\,(310\ \text{nm}) = 0.0042 \cdot [\text{MLE}](\text{mg/L}) + 0.0446$; R= 0.9908 (3)

MLE in THF (100-5000 mg/L): $\text{Abs}\,(665\ \text{nm}) = 0.0001 \cdot [\text{MLE}](\text{mg/L}) + 0.0046$; R= 0.9944 (4)

2.5 MLE release in a saline medium

The release of the active substance from each polymer was studied by immersing ca. 90 mg of impregnated polymer in 5 mL of phosphate buffer saline (PBS) at pH 7.4, simulating a body fluid. The release was quantified along 600 h according to the calibration line shown in equation 5.

MLE in PBS (1-70 mg/L): $\text{Abs}\,(280\ \text{nm}) = 0.0073 \cdot [\text{MLE}](\text{mg/L}) - 0.0074$; R= 0.9996 (5)

2.6 Antioxidant capacity of the MLE released in a saline medium

Samples of both impregnated polymers (~ 20 mg) were submerged in PBS for 240 and 480 hours. The antioxidant capacity of the released MLE was evaluated in the same way as described for the extract (section 2.2). In this case, the PBS solution was used as a control of the reaction.

2.7 Statistical analysis

All experiments were performed in duplicate and the results show the mean and the standard deviation in the error bars. To find significant differences between groups of results, simple variance analysis studies were carried out using Fisher's Least Significant Difference (LSD) procedure.

3. Results and discussion

The mango extract obtained using ethanol and CO_2 at high pressures showed an average concentration of 48 g/L. Calculated IC_{50} was 9.37 mg/L and the AAI was 2.47. According to Scherer and Godoy's classification (Scherer and Godoy, 2009), it was an extract with a very high antioxidant power (AAI>2). Other authors have obtained mango leaf extracts with comparative antioxidant properties, for example, Itoh and coworkers (Itoh et al, 2020) obtained a methanolic mango leaf extract with an IC_{50} of 9 mg/L.

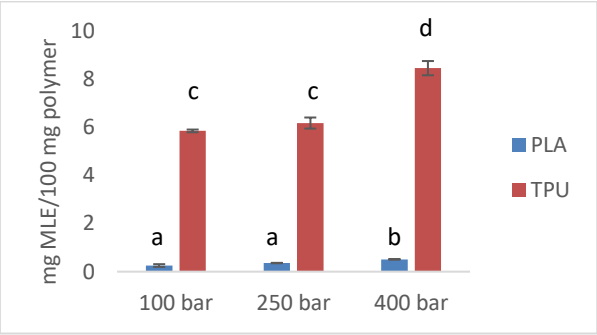

Figure 1: Comparative MLE impregnation loading in PLA and TPU at different working pressures. Different letters at the top of the bars (a,b,c,d) means significative differences between the groups with a 95% confidence level (p<0.05).

Regarding the impregnation, Figure 1 shows the quantification of MLE impregnated by SSI in the two polymers at different working pressures. The MLE load depends on the complex interrelationships between CO_2 in the supercritical state, the polymer, and the extract. First of all, a higher loading is observed in the case of TPU, which is on the order of 10 times higher than that of PLA. The difference in these results may lie both in the structure and the composition of the polymers, determining a better penetration of the extract molecules and better chemical interactions between extract and polymer. Machado and coworkers (Machado et al., 2022) estimated the swelling degree of these two polymers during de supercritical impregnation. They found a larger swelling in the TPU than in the PLA, which could explain why the MLE molecules can diffuse better in the expanded structure of the TPU, producing an increase in the impregnation yield. Regarding the chemical interactions, the MLE is a complex mixture with a lot of different compounds mostly represented by mangiferin

(Figure 2a), which has many hydroxyl groups that could interact via H-bonds with the carbonyl groups of both polymers (Figures 2b and 2c). Furthermore, in the case of TPU, -NH groups could form H-bonds with carbonyl groups of mangiferin, and interactions π-stacking can also arise between the aromatic rings of polyphenols and TPU, favoring the load in this polymer. Considering the operational conditions, the MLE impregnated improved when increasing pressure for both polymers, obtaining the best results at 400 bar. Moreover, Machado and coworkers (Machado et al., 2022) found a larger swelling in both polymers when pressure increased, which can be led to higher CO_2 sorption by the polymers. Besides, when increasing pressure, the solubility of substances in supercritical CO_2 could raises too (Méndez-Santiago and Teja, 1999), which could also promote the MLE loading.

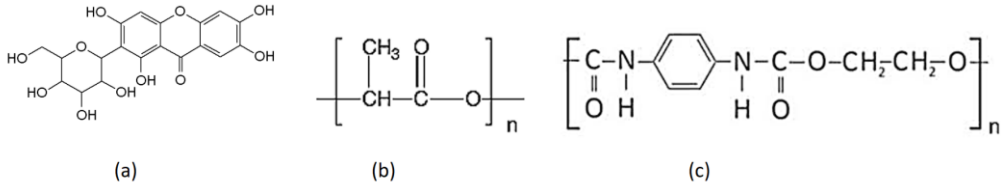

Figure 2: Chemical structure of (a) mangiferin, (b) polylactic acid (PLA), and (c) thermoplastic polyurethane (TPU).

The ultimate goal of these impregnated polymers would be their use as implants with controlled release of an extract with pharmacoactive capacities, so it is very important to know how the substance of interest is released. In this case, an *in vitro* release has been made in a saline medium and the results are shown in Figure 3.

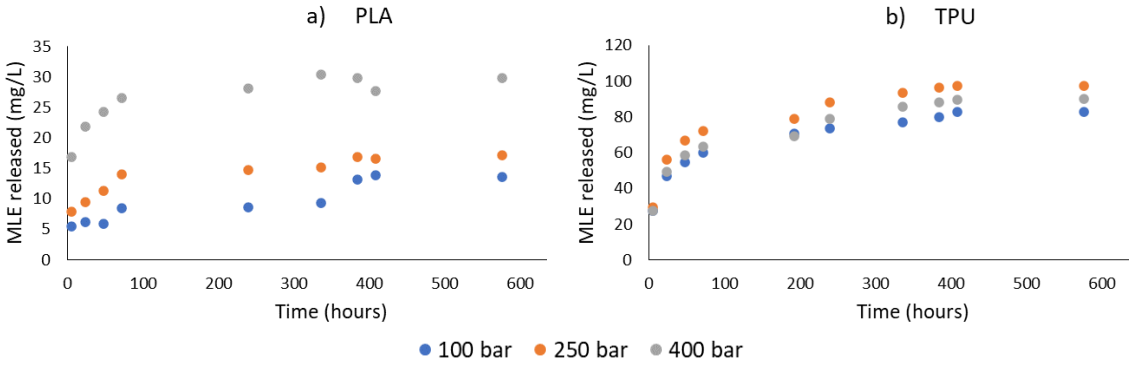

Figure 3: MLE released into a saline medium from impregnated polymer at different conditions.

It can be observed that the higher the loading, the greater the release of MLE. Regarding pressure conditions, it was observed that the polymers with higher loading (upper pressures) release a greater amount of extract into the medium, at each studied time. Possibly, higher impregnation pressures drove a larger swelling and a greater porosity by the polymer (Machado et al, 2022), so the contact surface with the medium would also be greater, allowing easier solubilization of the extract.

Regarding the kind of polymer, in the case of PLA (Figure 3a), a rapid initial MLE release was produced, stabilizing 336 h after starting, reaching a maximum concentration that remained constant until the end of the assay. On the contrary, the MLE release from the TPU seemed more progressive (Figure 3b). There was also an initial fast release followed by a slower release, but this release increased progressively.

In the case of PLA, the maximum MLE released corresponds to about a third of the total impregnated MLE, which indicates a remaining MLE inside the polymer. On the other hand, although the quantity of MLE released from TPU was higher than that from PLA, only 6-8 % of the total impregnated MLE migrated to the simulated medium. In this sense, it would be necessary to increase the test time to approximate a release model that considers the whole delivery of the extract. These were therefore the initial stages of release, and it is necessary to reach stages in which the substances that have become impregnated in the innermost structures of the polymer can be released into the environment, while the degradation of the polymers occurs (Verano-Naranjo et al, 2021). Another annotation according to these percentages of total impregnated MLE solved in the saline medium is that the release from the TPU is more slowly than from the PLA, maybe because the degradation of the polymer is also slower.

Figure 4 shows the antioxidant activity of the release medium at two points of the releasing experiment. First of all, it should be noted that, in all cases, at higher impregnation pressure the antioxidant activity was greater. As

discussed above, the amount of impregnated MLE increased with pressure, likewise the amount of extract with antioxidants compounds released into the medium.
Comparing both times for each of the polymers, it was observed that the longer the test lasted, the greater the amount of extract released and therefore the antioxidant capacity also increased. This increase was not very pronounced and the differences between the same experiments realized at the same conditions but at different times, in all cases, are not significant. This may be because, at the times when this experiment was carried out, the release was in the slow stage (see figures 3a and 3b), with little difference in the concentrations of the extract released.
Finally, comparing both polymers, in all cases the antioxidant capacity of TPU was greater than that of PLA. The impregnation loads for TPU were much higher than that for PLA and then the amount of MLE released into the medium from TPU was also larger than from PLA, however, its antioxidant capacity was not so different. The release of the compounds that form the MLE is conditioned by their affinity with the release medium. Thus, it is to be assumed that the most soluble compounds will be released first, leaving the compounds with the highest affinity with the polymer more retained. As explained above, phenolic compounds, which are those with the highest antioxidant capacity, may have a greater affinity with TPU than with PLA. Thus, although the amount of MLE released from TPU was greater than from PLA, perhaps the compounds released were not those with the greatest antioxidant capacity.

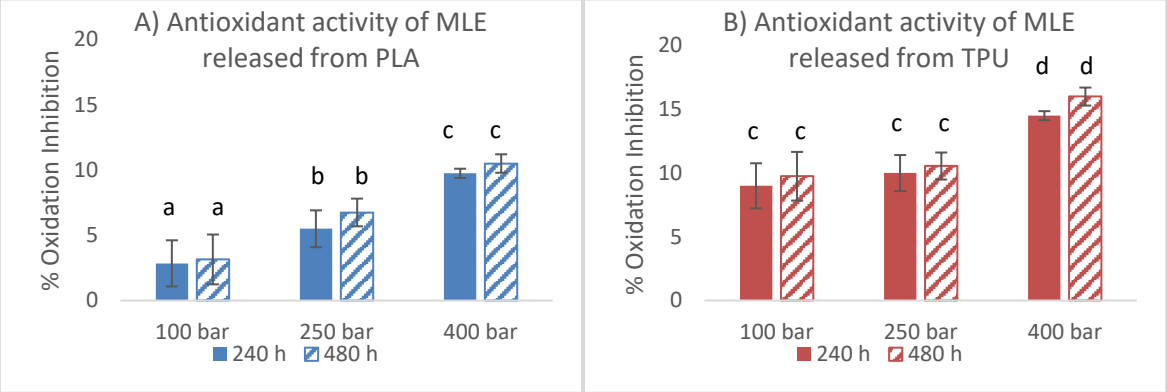

Figure 4: Antioxidant activity of the MLE released from PLA (A) and TPU (B) up to 240 and 480 hours. Different letters at the top of the bars (a,b,c,d) means significative differences between the groups with a 95% confidence level ($p<0.05$).

4. Conclusions

The analysis of the results obtained in this work revealed the potential of PLA and TPU polymers and the impregnation techniques with supercritical fluids in the biomedical field. On the other hand, the antioxidant characteristics of the extract demonstrated the relevance of raw materials that are discarded from other activities, such as mango leaves.
A greater number of studies on lactic acids make PLA the most widely used polymer today in biomedical applications. However, the differences in the physicochemical properties among polymers make the study of others for biomedical applications interesting. In fact, in this study, TPU polymers have been shown to have a higher load capacity than PLA polymers. Concerning the impregnation conditions, it was verified that the highest loads are obtained when using higher pressures in the impregnation for both polymers.
Considering the release of the active compound from the polymers, a faster initial release was observed in both polymers, followed by a slower release. In terms of the percentage released from its load, in PLA it was 30% compared to 8% in TPU.
Finally, with the bioactive tests it was shown that the impregnated polymers have antioxidant capacity, increasing this as the impregnated compounds are released.
Although the future of these materials seems promising, more studies are needed to evaluate the release mechanism of antioxidant compounds from these polymers. As well as mechanical tests that ensure the necessary quality of the polymers after supercritical treatment for their application in the manufacture of biomedical materials. In addition, assays are also required to ensure cell viability and in vivo performance of these mango leaf extract-impregnated polymers.

Acknowledgments

This publication is part of the R+D+i project reference PID2020-116229RB-I00, funded by MCIN/AEI /10.13039/501100011033/ and “ERDF A way of making Europe”. Some of the infrastructures used in carrying out this research have been funded by MCIN/AEI /10.13039/501100011033/, project reference EQC2019-005988-P. Thanks to the Institute of Subtropical and Mediterranean Horticulture “La Mayora” for providing the mango leaves.

References

Du, S., Liu, H., Lei, T., Xie, X., Wang, H., He, X., Tong, R., Wang, Y. 2018. Mangiferin: An effective therapeutic agent against several disorders (Review). Molecular Medicine Reports, 18(6), 4775-4786.

Fernández-Ponce, M.T., Casas, L., Mantell, C., Martínez de la Ossa, E.J. 2013. Potential Use of Mango Leaves Extracts Obtained by High Pressure Technologies in Cosmetic, Pharmaceutics and Food Industries. Chemical Engineering Transactions, 32, 1147-1152.

Ferreira, S., Araujo, T., Souza, N., Rodrigues, L., Lisboa, H. M., Pasquali, M., Trindade, G., Rocha, A. P., 2019, Physicochemical, morphological and antioxidant properties of spray-dried mango kernel starch. Journal of Agriculture and Food Research, 1, 100012.

Itoh, K., Matsukawa, T., Okamoto, M., Minami, K., Tomohiro, N., Shimizu K., Kajiyama, S., Endo, Y., Matsuda, H., Shigeoka, S. 2020. In vitro Antioxidant Activity of Mangifera indica Leaf Extracts. Journal of Plant Studies, 9, 2, 39-45.

Kumar, S., Hussain, A., Sansi, M. S., Iram, D., Priyanka, Vats, A., 2022, Antioxidative agents from medicinal plants. Chapter In: Research Anthology on Recent Advancements in Ethnopharmacology and Nutraceuticals, IGI Global, 179-194.

Liparoti, S., Franco, P., Pantani, R., De Marco, I., 2022. Supercritical CO_2 impregnation of caffeine in biopolymer films to produce anti-cellulite devices. The Journal of Supercritical Fluids, 179, 105411.

Liu, S., Qin, S., He, M., Zhou, M.,, Qin, Q., Wang, H. 2020. Current applications of poly(lactic acid) composites in tissue engineering and drug delivery. Composites Part B: Engineering, 199, 108238.

Machado, N. D., Cejudo-Bastante, C., Goñi, M. L., Gañán, N. A., Casas-Cardoso, L., Mantell-Serrano, C. 2022. Screening of the Supercritical Impregnation of Olea europaea Leaves Extract into Filaments of Thermoplastic Polyurethane (TPU) and Polylactic Acid (PLA) Intended for Biomedical Applications. Antioxidants, 11(6), 1170.

Mei, S., Ma, H., Chen, X. 2021. Anticancer and anti-inflammatory properties of mangiferin: A review of its molecular mechanisms. Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 149, 111997.

Méndez-Santiago, J., Teja, A.S. 1999. The solubility of solids in supercritical fluids. Fluid Phase Equilibria, 158–160, 501-510

Pratelli, G., Carlisi, D., D’Anneo, A., Maggio, A., Emanuele, S., Palumbo Piccionello, A., Giuliano, M., De Blasio, A., Calvaruso, G., & Lauricella, M. 2022. Bio-waste products of Mangifera indica L. reduce adipogenesis and exert antioxidant effects on 3T3-L1 cells. Antioxidants, 11(2), 363.

Rivera, D. G., Balmaseda, I. H., León, A. A., Hernández, B. C., Montiel, L. M., Garrido, G. G., Cuzzocrea, S., Hernández, R. D., 2006. Anti-allergic properties of Mangifera indica L. extract (Vimang) and contribution of its glucosylxanthone mangiferin. The Journal of Pharmacy and Pharmacology, 58(3), 385-392.

Saha, S., Sadhukhan, P., Sil, P. C., 2016, Mangiferin: A xanthonoid with multipotent anti-inflammatory potential: Therapeutic Applications of Mangiferin. BioFactors, 42(5), 459-474.

Sánchez-Gomar, I., Benítez-Camacho, J., Cejudo-Bastante, C., Casas, L., Moreno-Luna, R., Mantell, C., Durán-Ruiz, M. C., 2022. Pro-Angiogenic Effects of Natural Antioxidants Extracted from Mango Leaf, Olive Leaf and Red Grape Pomace over Endothelial Colony-Forming Cells. Antioxidants, 11(5), 851.

Scherer, R., Godoy, H. T., 2009, Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chemistry, 112(3), 654–658.

Verano-Naranjo, L., Cejudo-Bastante, C., Casas, L., Mantell, C., Martínez De La Ossa, E.J., 2021, In vitro study of the release of drugs impregnated by supercritical technology in polylactic acid for biomedical applications. Chemical Engineering Transactions, 86, 1063–1068

Zalepugin, D. Y., Tilkunova, N. A., Chernyshova, I. V. 2020. Impregnation of polymer materials in supercritical media (a review). Russian Journal of Physical Chemistry B, 14(7), 1067-1076

Zdrahala, R.J., Zdrahala, I.J. 1999. Biomedical applications of polyurethanes: a review of past promises, present realities, and a vibrant future. Journal of biomaterials applications, 14, 67-90.M’Bengue M-S, Mesnard T, Chai F, Maton M, Gaucher V, Tabary N, García-Fernandez M-J, Sobocinski J, Martel B, Blanchemain N. 2023. Evaluation of a Medical Grade Thermoplastic Polyurethane for the Manufacture of an Implantable Medical Device: The Impact of FDM 3D-Printing and Gamma Sterilization. Pharmaceutics, 15(2):456.