AN ENVIRONMENTALLY FRIENDLY APPROACH OF EXTRACTION AND CHARACTERIZATION OF NATURAL PRODUCTS: A CONCISE REVIEW

Duarte, G. C. A.[1*]; Carvalho, E. M[2]; Machado, T. B.[1]; Santos, W. C.[1*]
[1]*UFF/Pharmacy Faculty/ Niterói, RJ, Brazil*
[2]*FIOCRUZ/Farmanguinhos/Rio de Janeiro, RJ, Brazil*
**glaucecad@id.uff.br, wsantos@id.uff.br*

Introduction

Since the beginning of time, natural products (NP) are used as medicine by humanity and represent about 62% of approved drugs (NEWMAN; CRAGG, 2016). Conventional methods used for extraction (maceration, Soxhlet) and identification (High-Pressure Liquid Chromatography – HPLC) of bioactive compounds (BC) are laborious and time-consuming. Therefore, in the last decades, non-conventional techniques have been developed to increase yield and quality of extract, reduce operational time, and mainly avoid using organic solvents, bringing an environmentally correct approach. (AZMIR *et al*., 2013).

The present study presents a concise systematic review of non-conventional techniques of extraction and characterization of NP comparing their advantages and disadvantages with conventional methods.

Method

The keywords non-conventional extraction methods, NP, characterization methods, ultrasound-assisted extraction, NMR spectroscopy, metabolomics, chemometrics as characterization techniques were used on the *Periodicos Capes* database to do this research. Throughout the study, articles published in the last decade were taken into account.

Results / Discussion

Nowadays, green techniques have many novelties under study. This work focused on the new extraction methods of BC and in the characterization by NMR spectroscopy. Conventional extraction techniques for BC involve the use of solvents coupled with heat and/or shaking, such as Soxhlet extraction and maceration, respectively. Maceration is widely used but consumes a lot of bench time and solvent volume and needs shaking to increase the yield. Soxhlet extraction allows only the extraction of small amounts at a time and uses high temperatures (MOHAMMADPOUR *et al*., 2019). Non-conventional extraction techniques seek to enrich the classical methods and promise the reduction of environmental impact, decrease processing times, and reduce solvents. One mostly used technique of extraction, the UAE, a type of sound wave at a frequency above the threshold of human hearing from 20 to 100 MHz, produces a phenomenon with production, growth, and collapse of bubbles (cavitation). This phenomenon induces diffusion across the cell wall and washes the contents of cells after breaking the barriers. The advantages of UAE include high reproducibility of assays, low solvent consumption, and shorter process time (RAMOS-HERNÁNDEZ *et al*., 2018;PRAKASH MARAN *et al*., 2017). Traditionally, HPLC is the first-choice technique for BC quantification. However, this technique requires known standards, a large volume of solvent and residue, lengthy sample preparation, and development of the method according to the type of sample (SNYDER *et al.*, 2010). On the other hand, NMR spectroscopy, which has been used only to

characterize bioactive compounds, is currently being applied in the quantification of metabolites and quality control of herbal medicines. As advantages, NMR spectroscopy has minimal sample preparation, total sample recovery, the ability to quantify metabolite levels with an internal standard of known purity unrelated to the analyte, a high level of experimental reproducibility, and an inherently non-destructive nature of NMR spectroscopy (NETO, 2012). These advantages have made the NMR technique a powerful tool for metabolomics studies. The NMR-based metabolomics flux could be performed without prior chromatographic separation or pre-treatment. This methodology consists of four steps: preparation of the samples, acquisition and processing of the spectra, multivariate statistical analysis (chemometrics), and research of metabolites in databases (CANUTO *et al*., 2018) (FIGURE 1).

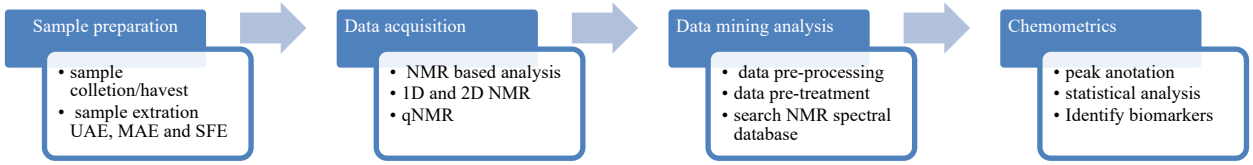

Figure 1. Critical stages of a plant metabolomics study.

Our reviewed show that UAE increases yield by at least 20-25%, extraction rates ten times more rapid, and decrease in the amount of solvent by at least ten times (MOHAMMADPOUR *et al*., 2019). NMR-based metabolomics has a significant advantage in the characterization and quantification of BC. It avoids the isolation steps at at least seven complex and laborious sample processing, such as fractionation and purification. Besides that, crude extract analysis allows a more reliable study addressing the synergistic effects between the substances contained therein (CASANOVA *et al.*, 2017). NMR spectroscopy, also, exhibits essential characteristics (exploited extensively in numerous studies) as the capability to analyze intact tissues, identify unknown metabolites, and trace metabolic pathway atoms. In parallel, the NMR spectroscopy had significant progress in the latest decade instrumentation, method developments, databases, and automation focused on higher throughput and overcoming the limitations of NMR, particularly resolution and sensitivity.

Conclusion

Non-conventional extraction and characterization techniques have significant advantages over traditional ones about the yield and quality of extracts, time of analysis, sample preparation to obtaining the results; decrease in systematic errors; reduction of the volume of solvents used and residues generated. Therefore, they can be considered green techniques and essential tools in the study of NP.

Acknowledgments

FAPERJ (Emergency Support for Stricto Sensu Graduate Programs and Courses in the State of Rio de Janeiro Project E -26/200.930/2017) and CAPES (Finance Code 001).

Bibliographic References

AZMIR, J. *et al*. 2013. *Journal of Food Engineering*, 117(4):426–436, 2013.
CANUTO, G. A. B. *et al*. 2018. *Quimica Nova*, 41(1):75–91.
CASANOVA, L. M.; COSTA, S. S. 2017. *Revista Virtual de Quimica*, 9(2):575–595.
CHEMAT, S. *et al*. 2017. *Ultrasonics Sonochemistry*, 34:310–316.
MOHAMMADPOUR, H. *et al*. 2019. *Industrial Crops and Products*, 131:106–116.
NETO, F. F. G. *Tratamento Metrológico e Quimiométrico de Dados de Ressonância Magnética Nuclear para Amostras de Biodiesel em Diesel*. 140 p. - Universidade Federal de São Carlos, 2012.
PRAKASH MARAN, J. *et al*. 2017. *Arabian Journal of Chemistry*, 10:S1145–S1157.
RAMOS-HERNÁNDEZ, J. A. *et al*. 2018. *Journal of Food Science and Technology*, 55(7):2377–2383.
SNYDER, L. R.; KIRKLAND, J. J.; DOLAN, J. W. **Introduction to Modern Liquid Chromatography**. Third ed. New Jersey: John Wiley & Sons, Inc, 2010. 957 p.