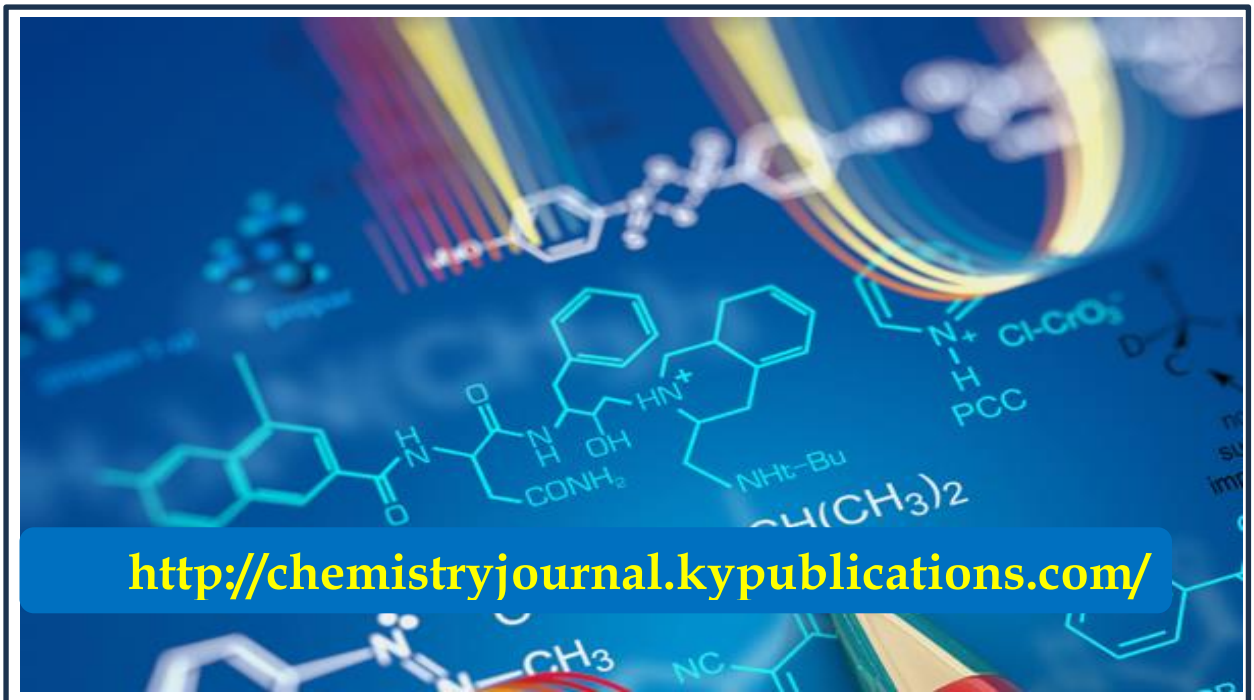

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Research Article

Extraction of secondary metabolites from the *Maytenus emarginata* plant for identification using high-performance liquid chromatography

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ABSTRACT

The aim of the work was to develop and validate extraction methods for the detection of secondary metabolites by HPLC, from the young Kankera plant (*Maytenus emarginata*). In the laboratory, the seeds were placed in a germination box and taken to the germination chamber. After 18 days of incubation, the seedlings and cotyledons were separated from the germinated seeds and placed in an ultra-freezer at (-34 ^{o}C) and subsequently freeze-dried. The samples were separated into parts for extraction by maceration, infusion and ultrasound in ultrapure water and 99% Ethanol. From the aliquot taken from the extract, it resulted that for seedlings the ultrasound method with water obtained higher chromatographic peaks with greater intensities and for cotyledons it was infusion with water. Thus, it is concluded that the ultrasound extraction method with water is more efficient for seedlings and the infusion extraction method with water is more efficient for the cotyledon, compared to the other methods tested in this work.
Keywords: Ultrasound, Infusion, HPLC.

Introduction

Among medicinal plants, Kankera (*Maytenus emarginata*), (Figure 1) is a species native to South India [4] and has been used for decades by popular medicine to treat various pathologies, due to its pharmacological potential promoted by secondary metabolites, such as phenolic compounds, terpenes and alkaloids, identified in studies previously carried out with leaf extracts. These compounds are naturally produced by plants in response to biotic or abiotic factors and are of high economic interest.

Knowing the importance of natural compounds, knowing the extraction methods is crucial, as the quantity and quality of the extract will depend on the extraction method used. According to Gori et al. (2021), extraction are methods used to remove the active fraction from a plant material, using appropriate liquids. Among the most used conventional extraction methods are: ultrasound, maceration and infusion. However, there are still no studies that determine the most effective method of extracting secondary metabolites from the young plant.

Thus, the aim of the work was to develop and validate extraction methods for the detection of secondary metabolites by high-performance liquid chromatography from the young Kankera plant.

Materials and methods

In the laboratory, the seeds of Kankera (*Maytenus emarginata*) were placed in a gerbox 11 × 11 cm, containing a substrate with vermiculite and moistened with 40 mL distilled water, each plate. After sowing, the seeds were taken to a germination chamber, with a 12-hour light and 12-hour dark photoperiod with a constant temperature of 27 °C. After 18 days of incubation, seedlings and cotyledons were separated from the germinated seeds and kept in an ultrafreezer at (-34 ^{o}C). then lyophilization was carried out using the Freeze dryer lyophilizer.

With the freeze-dried samples, they were divided into parts to obtain extractions by Infusion, Maceration and Ultrasound. For each extraction method used, a sample was separated to extract in water (m/v) and the same sample to extract in 99% ethanol, following the methodology described.

Figure 1: *Maytenus emarginata* (Willd.) (Kankera) plant

For ultrasound, the methodology described by Sandhu, (2021) was used where the cotyledon and seedling samples were kept agitated by the probe for one hour with a maximum temperature of 40 ^{o}C. During infusion, the solvents were heated to around 60^{o}C and each sample was kept stirring in the dark for two hours, with temperatures varying between 60 and 70^{o}C (OLIVEIRA, 2016). During maceration, the solvents were not heated and the samples were stirred for 24 hours at room temperature, in the dark (FERREIRA, 2020). After extractions, the samples were diluted, filtered and analyzed on HPLC, according to the laboratory's own protocol.

Results and discussion

In this work, the HPLC device was used, a separation technique based on the distribution of the components of a mixture between two immiscible phases, the liquid mobile phase and the solid stationary phase, contained in a cylindrical column. The retention time of the sample analyzed in this column varies according to the interactions of the substances present in the sample with the stationary and mobile phases, therefore, each compound was eliminated from the column and identified by the detector at a different time, generating a chromatographic peak. The analysis of the different peaks allowed us to study the substances present in the sample, as shown in the images below.

Figure 2 shows the chromatogram of the Kankera seedling extract, obtained only with the ultrasound method with water:

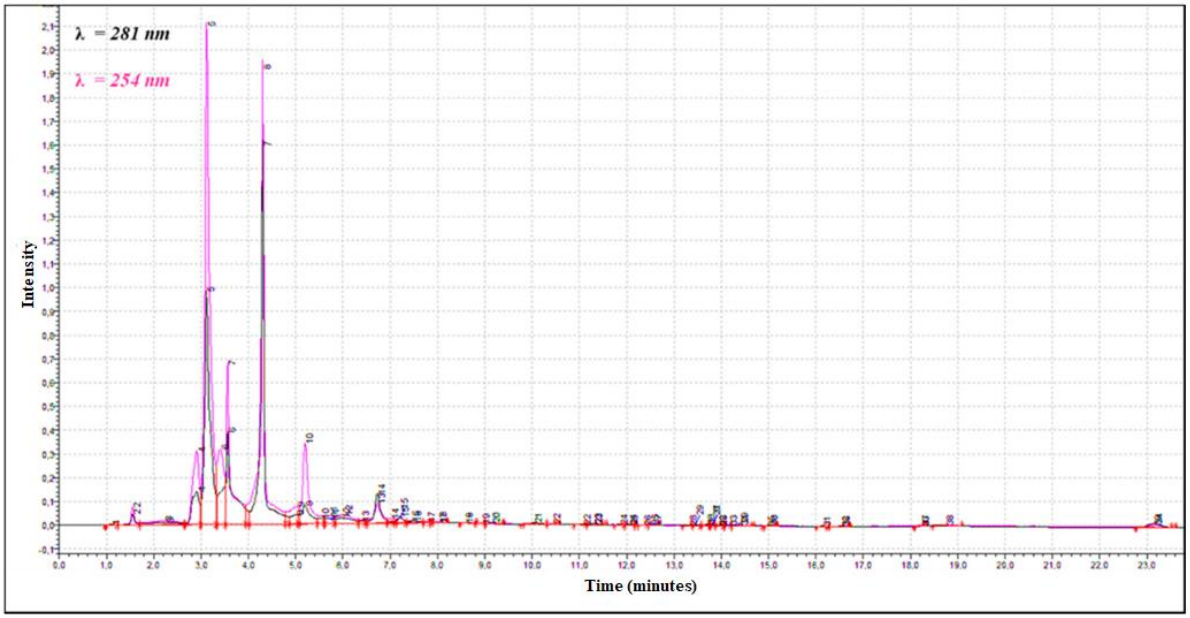

Figure 2: Chromatogram obtained by HPLC-DAD of the Kankera Seedling extract Ultrasound Water, at 281 nm, 254 nm.

As an analytical response to the Seedling extracts, a number of larger chromatographic peaks were obtained in the samples extracted with water, both for the Infusion (30 peaks) and Maceration (27 peaks) extraction method (both images not shown) and also in the Ultrasound method (38 peaks) (Figure 1), at a wavelength of 281 nm. Another important fact is that in samples extracted with water regardless of the method, the peaks had greater intensities, reaching close to 100 mAU in Infusion and Maceration and 210 mAU in Ultrasound. In the samples extracted with ethanol (images not shown), the intensities were 30 mAU, 20 mAU and 70 mAU, for Infusion, Maceration and Ultrasound, respectively.

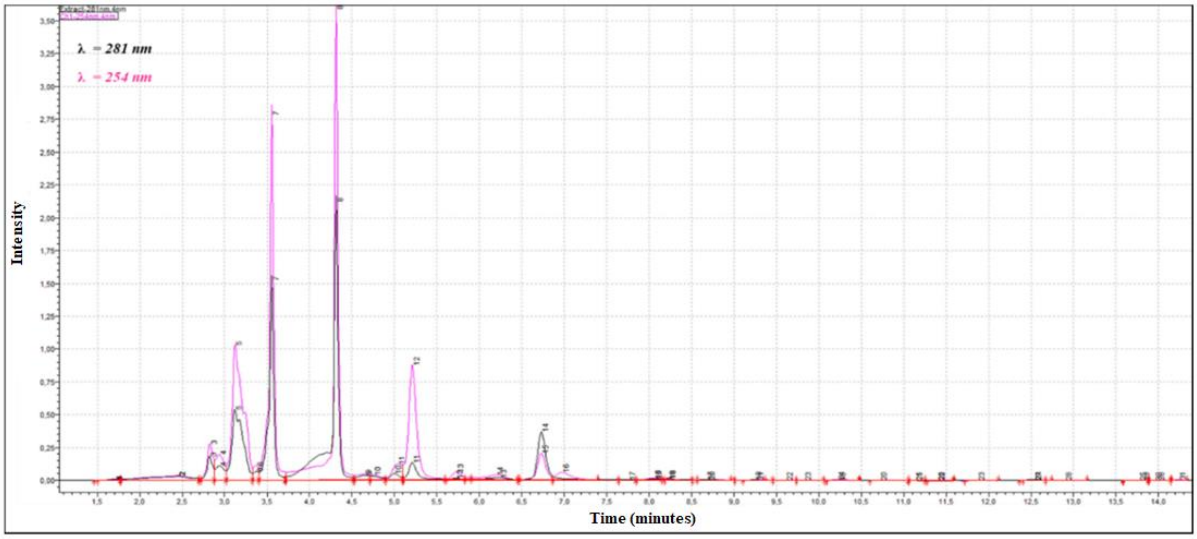

Figure 3 - Chromatogram obtained by HPLC-DAD of the Kankera Infusion Water Cotyledon extract at 281 nm and 254 nm.

The analytical responses of the Cotyledon extracts, a number of larger chromatographic peaks were obtained in the samples extracted with water also, both for the Infusion extraction method and in maceration (image not shown), at a wavelength of 254 nm. However, the intensities of the peaks were higher in the Infusion with water extraction method, reaching 350 mAU (Figure 3). These molecules

that presented the peak with greater intensity have a retention time in the column of 4.3 minutes, that is, they came out during the analysis time when the proportion of water was higher (80%). This indicates that these have a more polar character and also have a response at a wavelength of 281nm. Thus, for the cotyledon, the extraction method that apparently showed the best responses was not Ultrasound but Infusion.

Conclusions

From the results, it can be concluded that the most effective extraction method for the Kankera seedling was ultrasound and for the cotyledon, infusion, both with water as solvent.

References

[1]. Daniele F. Ferreira, Bruna N. Lucas, Monica Voss, Daniel Santos, Paola A. Mello, Roger Wagner, Giancarlo Cravotto, Juliano S. Barin, Solvent-free simultaneous extraction of volatile and non-volatile antioxidants from rosemary (*Rosmarinus officinalis* L.) by microwave hydrodiffusion and gravity, *Industrial Crops and Products*, Volume 145, 2020,112094

[2]. Gori, A.; Boucherle, B.; Rey, A.; Rome, M.; Fuzzati, N.; Peuchmaur, M. Development of an innovative maceration technique to optimize extraction and phase partition of natural products. *Fitoterapia*. 148 (2021) 104798.

[3]. Khichar, M. K., Sharma, M., & Agrawal, R. D. (2019). Maytenus emarginata: A Folklore Ethnomedicinal Plant of Rajasthan. *Journal of Drug Delivery and Therapeutics*, 9(4-A), 901-907.

[4]. Oliveira, V. B. et al., Efeito de diferentes técnicas extrativas no rendimento, atividade antioxidante, doseamentos totais e no perfil por clae- dad de dicksonia sellowiana (presl.). Hook, dicksoniaceae. *Revista Brasileira de Plantas Medicinais*, *Campinas,* v. 18, n.1, supl. I, p. 230 – 239, 2016.

[5]. Sandhu, H. K., et al., Effect of Ultrasound-Assisted Pretreatment on Extraction Efficiency of Essential Oil and Bioactive Compounds from CitrusWaste By-Products. *Separations*, Vol. 8, page 244, 2021.