# REVIEWING THE OPTIMAL CONDITIONS FOR EFFICIENTLY HARNESSING THE PHYTOTHERAPEUTIC POTENTIAL OF THE INVASIVE *CARPOBROTUS EDULIS* (L.) N.E. BR PLANT THREATENING PLANT DIVERSITY

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**ÖZET**

Bu çalışma kapsamında Hottentot-fig olarak bilinen istilacı ve diğer bitki türlerini tehdit eden *Carpobrotus edulis* (L.) N.E.Br bitkisi incelenmektedir. Bu bitki istilacı özelliği ile kıyı bölgelerinde biyolojik çeşitlilik adına büyük bir tehdit oluşturmaktadır. Literatürdeki birçok çalışma bu kriz durumunu fırsata çevirmek adına Hottentot-fig bitkisinin fitoterapötik potansiyellerini ortaya koymayı hedeflemişlerdir. Bitkinin hasat zamanı ve yerine göre değişen biyoaktif bileşenleri ve antioksidan, antiinflamatuar özellikleri değerlendirilerek bu bitkinin gıda ve nutrasötik endüstrilerde kullanılabilirliği ortaya konulmuştur. Bu çalışmalarda uygun metodoloji ve en verimli sonuçları elde etmek için optimum parametreleri belirlemek adına diğer literatür çalışmaları ile ekstraksiyon, toplam fenolik içerik

HPLC-DAD gibi yöntemlerin sonuçları göz önünde bulundurularak kıyaslamakar yapılmaktadır. Buna bağlı olarak çalışmaların eksik ve güçlü yanları belirlenmiştir. Bizim çalışmamız kapsamında, literatürde kullanılan ve bilinen yöntemlerin yanı sıra alternatif yöntemlerin de göz önünde bulundurulması gerektiği, bu yöntemlerin sonuçlarının incelenmesi gerektiği ve farklı metot stratejileri ile verimli sonuçlar elde edilebileceği belirtilmektedir.

**Anahtar Kelimeler:** *Carpobrotus edulis* (L.) N.E.Br, Hottentot-fig, fitoterapötik, biyoaktif bileşen, yöntem, istilacı tür.

**ABSTRACT**

This study investigates the *Carpobrotus edulis* (L.) N.E. Br plant, known as Hottentot-fig, which is invasive and threatens other plant species. This plant poses a great threat to biodiversity in coastal areas with its invasive feature. Many studies in the literature have aimed to demonstrate the phytotherapeutic potentials of the Hottentot-fig plant as a way to turn this crisis situation into an opportunity. By evaluating the bioactive components and antioxidant and anti-inflammatory properties of the plant, which vary depending on harvest time and location, the usability of this plant in the food and nutraceutical industries has been demonstrated. In these studies, comparisons are made with other literature studies to determine the optimum parameters for the

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most efficient results, taking into account the outcomes of methods such as extraction, total phenolic content, and HPLC-DAD. Consequently, the strengths and weaknesses of the studies have been identified. Within the scope of our study, it is stated that alternative methods should be taken into consideration in addition to the known methods used in the literature, the results of these methods should be examined, and efficient results can be obtained with different method strategies.

**Keywords:** *Carpobrotus edulis* (L.) N.E. Br, Hottentot-fig, phytotherapeutic, bioactive compound, method, invasive species.

## 1. INTRODUCTION

*Carpobrotus edulis* (L.) N.E. Br, commonly known as Hottentot-fig, is a species of plant belonging to the Aizoaceae family, characterized by broad leaves and yellow flowers. This species is designated as problematic due to its invasive nature in coastal areas. Hottentot-fig contains flavonoids such as rutin, catechin, and hyperoside, which confer anti-bacterial properties to the plant [1]. However, attention should be paid to harvesting this plant in the regions and during the seasonal times that would yield the most efficient anti-bacterial properties from its flavonoids. In this study, extracts obtained by harvesting this plant species at different locations and times of the year were compared for the yield of polyphenols [2]. For instance, the extracts of Hottentot-fig exhibit stronger anti-inflammatory and antioxidant properties during summer and spring months compared to winter months [1].

The *Carpobrotus edulis (L.)* N.E. Br species examined within the scope of this article, known as Hottentot-fig, is considered problematic due to its invasive nature in coastal areas. Coastal environments act as a transition zone between land and sea and provide protection against disasters such as floods and storms [3]. However, these areas cannot defend themselves against natural or anthropogenic changes. Management of Hottentot fig species that invade these areas is challenging as they inhibit the growth of other species and suppress native species. This situation causes various disadvantages in terms of biodiversity of the ecosystem and other species. Turning such crises into opportunities is of great importance for both ecosystems and humanity [2]. For example, researching and discovering the biochemical properties of the Hottentot fig and using these discovered properties beneficially for living organisms may be the most appropriate strategy to combat such invasive species [3]. *Carpobrotus edulis* (L.) N.E. Br is a succulent plant that can grow to a diameter of at least 50 m. It can live in Mediterranean countries and South Africa. Characterized by its rapid growth, the Hottentot fig acts as an invasive species, occupying the places of native plant species in coastal areas such as sand dunes and sea cliffs [1].

This plant species contains a rich array of bioactive compounds, such as flavonoids, phenolic acids, triterpenes, and alkaloids. Bioactive compounds are substances that exhibit physiological or cellular activities, particularly those with positive implications for health[2]. Flavonoids, as an example of bioactive compounds, are commonly found in plants and possess antioxidant properties, making them potential candidates for pharmaceutical and medical purposes [4]. Phenolic acids, on the other hand, are present not only in plants but also in foods such as fruits and vegetables [5]. They belong to the phenolic group and exhibit properties such as antimicrobial, anti-inflammatory, and antioxidant effects. Triterpenes, falling into the chemical class of isoprenoids, play roles in tasks like antimicrobial functions and the regulation of hormonal metabolism in plants [6]. Alkaloids, on the other hand, belong to the group of organic chemical compounds containing nitrogen in their structures [7]. While they contribute to the defense mechanisms of plants against harmful organisms, they are also utilized in medicine, such as in the case of morphine, due to their analgesic properties [8]. However, their usage can have both beneficial and harmful effects, depending on the context [1,2].

The fact that *Carpobrotus edulis* contains flavonoids and therefore antioxidants is an indication that it has the potential to be used in fields such as pharmacology. In addition to its antioxidant properties, this and other literature studies show that Hottentot-fig has bioactive properties that can be used in the field of medicine, such as anti-melanogenic, that is, equalizing skin tone and protecting skin health, wound healing, anti-bacterial and anti-fungal, thanks to the bioactive components it has. Due to these properties, it is an antiseptic for diabetes, tuberculosis, colds, eczema, burns, and a possible therapeutic drug even for HIV infections.

## 2. METHODS USED IN THE ANALYSIS OF THE HOTTENTOT-FIG PLANT

This study examines the methodologies of different literature studies involving the collection and processing of the Hottentot-fig plant, the selection of extraction stages, and profile analysis in terms of nutritional, chemical, and bioactivity aspects. Additionally, it will help analyze the most efficient methodological techniques based on the results obtained from these analyses of the plantIn the chemical selection stage, DPPH, ABTS, and NO radicals are utilized to measure the antioxidant capacity of the Hottentot-fig plant. Noui et al. Accordingly, gallic acid, quercetin, and catechin standards are used to determine the phenolic compounds found in plant samples [9]. For HPLC-DAD analysis, various commercial standards, and chemicals such as chlorogenic, coumaric, and gallic acids are used. All these chemicals are chosen for the determination of phenolic components in the plant and the quantification of their concentrations using the Folin-Ciocalteu (F–C) reagent [10].

The collection, processing, and extraction of the Hottentot-fig plant are essential steps in the study to utilize it. However, in this research, Hottentot-fig was collected in three different regions in Portugal and during different seasons, such as January, May, July, and November, to investigate the bioactive yield at different times and in different regions. For the extraction stage, the plant was dried at a temperature of 40 degrees Celsius. The reason for setting the temperature at 40 $^{o}$C could be to ensure the optimum drying temperature that does not harm the bioactive properties of the plant. The powdered form of the plant was extracted with a 80% water-acetone mixture for 24 hours [1, 11]. The choice of acetone and water as the solvent for extraction may be due to its ability to dissolve phenolic components present in the Hottentot-fig plant, making it the most suitable solvent for analyzing bioactive components. Extraction conditions, such as duration, extraction solution, and ratio, vary depending on the plant used, and the optimal conditions that provide maximum yield should be selected [12]. The continued process in the rotary evaporator is aimed at removing alcohol derived from acetone in the solution and increasing the concentration of components. Filtering the extracted solution helps remove solid particles, obtaining a purified solution. Dimethyl sulfoxide (DMSO) is used to dilute and store the obtained extracts and storing the samples at -20 $^{o}$C is ideal for long-term storage [13].

This study encompasses two separate sections for nutritional profile analysis: proximate composition and mineral composition [14]. The proximate composition is a crucial stage to analyze the components of the Hottentot-fig plant. It determines the total ash, moisture, crude carbohydrates, crude fat, and crude protein content in this plant species. In this article, the moisture content of Hottentot-fig was calculated based on the fresh weight of the samples, while ash content was determined using the ashing method. The Kjeldahl method was employed to determine crude protein, the Bligh-Dyer method for crude fat, and crude carbohydrates were calculated by subtracting the weights of other identified components [15]. The second section of nutritional profile analysis is mineral composition. This stage involves determining the macro and micro mineral content of plant samples. Macro minerals refer to large quantity minerals such as sodium and calcium, whereas micro minerals contain smaller amounts of minerals like copper and zinc [16]. For mineral analysis in this article, a Microwave Digestion System, Microwave Plasma - Atomic Emission Spectrometer were used. The purpose of employing acidic digestion might be to break down organic matter in plant samples to release minerals in free form. The use of low-pressure microwave systems may aim to enhance the efficiency of mineral release. The application of the Atomic Emission Spectrometer could be to obtain macro and micro elements in the mineral composition stage [17].

The chemical analysis of the Hottentot-fig plant involves several stages, including the determination of total phenolic content (TPC), total flavonoid content (TFC), condensed tannins determination, and High-Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD) for phenolic profile analysis. This stage allows for the quantification of total phenolic, flavonoid, and condensed tannins contents in previously obtained plant samples. TPC represents the total phenolic components in the plant sample, which, due to their antioxidant properties, is crucial in demonstrating the antioxidant capacity and bioactive nature of Hottentot-fig. The presence of phenolic components in a plant means the neutralization of reactive oxygen species, combating free radicals that negatively impact cells [18]. Similar to the TPC method, TFC is a technique used to determine the total flavonoid content in plant samples, and like phenolic compounds, flavonoids possess antioxidant properties, contributing to environmental protection and potential therapeutic roles in human health. The TFC method involves the use of $AlCl_3$ as a reagent, forming a color-changing complex upon reacting with flavonoids. The absorbance of the complex formed at a specific wavelength is measured to determine the quantity of flavonoids using standards [19]. Condensed tannins determination (CTC) is employed in this study to identify the tannin profile and bioactive properties, such as anti-inflammatory effects, of Hottentot-fig. High CTC content protects plants against other insects and plants, indicating high antioxidant and anti-inflammatory properties [1, 20]. Another chemical analysis method used is High-Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD) for phenolic profiling determination. With the HPLC-DAD system, the quantity of phenolic compounds present is measured [21]. Different wavelengths in the nanometer range are used in this stage to detect various phenolic components, as each wavelength corresponds to specific phenolic compounds. The selection of the HPLC-DAD method in this study may be attributed to its ability to separate complex phenolic compounds in plant samples, save time by analyzing multiple compounds simultaneously, and provide numerical results crucial in studies where precise measurements of phenolic compound quantities are essential[22].

In this study, the bioactive properties of the Hottentot-fig plant were analyzed for antioxidant and anti-inflammatory capacities in vitro. The EC50 values of the extracts were determined along with the activity percentage using diluted samples and negative controls to calculate the concentration ratio at which half-maximal effect is observed [23]. EC50 represents the concentration ratio at which half-maximal effect is observed for a compound. Experimental results revealed the concentration at which Hottentot-fig exhibited half-maximal antioxidant effect. A lower EC50 ratio at a low concentration indicates a higher antioxidant capacity [24]. DPPH, ABTS, and FRAP analysis methods were employed to analyze the antioxidant properties of the extracts using radical-based methods [25]. CCA and ICA analyses were conducted to detect the chelating properties of metal ions in the plant sample. The chelation property of metal ions in the plant indicates the ability to combat oxidative stress. In vitro anti-inflammatory activity analysis was performed to analyze the chemical components in the Hottentot-fig plant extract for anti-inflammatory bioactive properties. In this stage, the production of nitric oxide (NO) on LPS-stimulated macrophage cells was examined to assess the anti-inflammatory property. LPS stimulates macrophages due to its presence in the cell wall of bacteria, potentially triggering inflammatory responses by stimulating the immune system [1]. The examination of NO production serves as a good method to understand the inflammatory effect since an increase in NO production is observed in LPS-stimulated macrophages [26]. Measuring the production allows the determination of macrophage cell inflammatory levels [27]. If the bioactive components in the Hottentot-fig plant exhibit the ability to reduce or inhibit NO production, the anti-inflammatory property of the plant is established [28]. The Griess method was used in this stage to measure the amount of NO produced [29]. Excessive measurement of NO production indicates a response of cells to inflammation.

The final method used in the article is the statistical analysis stage. It is a crucial step for optimizing the results obtained during the experiments, accurately interpreting the data, and demonstrating the scientific validity of the study. In this research, the repetition of the experiments three times and the consistency of the results indicate the reliability of the study. XLSTAT and GraphPad Prism programs were employed to transform the obtained results into different visual forms, such as graphs, to interpret the results effectively. Additionally, in this study, the p-value calculated within the programs is used to determine the significance of the study [30]. If the p-value is less than 0.05, the study is considered statistically significant, and the results of the bioactive properties among Hottentot-fig plants collected from different regions and times are significantly different. Agglomerative Hierarchical Clustering (AHC) and Principal Component Analysis (PCA) are used to analyze the relationship in the experiments conducted on Hottentot-fig plants [31].

In summary, the material and method section of this article utilized various techniques to perform analyses aimed at determining the profiles of bioactive components and properties of Hottentot-fig plants at different times and regions. For instance, analyses such as DPPH, ABTS, NO radicals, TPC, TFC, and CTC were employed to assess the capacity of bioactive properties, such as antioxidants, in Hottentot-fig samples. In addition to these methods, different techniques, such as Oxygen Radical Absorbance Capacity, used in other studies in the literature, could also be applied. However, it is crucial to make the right choices, considering that the chemicals used in the analyses do not compromise the bioactive yield of the studied plant extract and that the materials in the laboratory are suitable. HPLC-DAD was used to identify the type and quantity of phenolic compounds in the plant sample. This method is highly sensitive and selective, making it more practical compared to other methods.

To determine anti-inflammatory activities, methods such as the stimulation of RAW 264.7 cells with LPS and the analysis of NO production were employed. Through this technique, the anti-inflammatory property of the Hottentot-fig plant was identified. The use of RAW 264.7 cells for determining the anti-inflammatory property might be due to its widespread application as a method to reveal anti-inflammatory characteristics. In summary, the application of various method techniques for each analysis in this article is a crucial step in discovering the potential bioactive properties, such as antioxidants and anti-inflammatory features, of the Hottentot-fig plant, which poses a threat to biodiversity due to its invasive nature in different regions and times. This exploration is significant for identifying the plant as a potential candidate for beneficial drug use in humanity.

## 3. DIFFERENCES ACCORDING TO ANALYSIS METHODS

When examining the results of the nutritional profile analysis in the study, it is observed that different outcomes are obtained when the Hottentog-fig plant is cultivated at different times and locations. During the fall and winter months, the ratios of components such as crude protein, fat, ash, and moisture are higher, whereas in the summer and spring months, fibers and carbohydrates are found in higher proportions. In addition to the seasonal variation in the components of the Hottentot-fig plant, the location also influences these components. The plant grown in Ria Alvor (A) region has a higher fiber content, while the one collected in Ancao beach (B) region has a higher carbohydrate content. Regarding minerals, it was determined that the Hottentot-fig plant in regions A and B has a high concentration of Ca, Na, and Fe, while in the Ria Formosa (C) region, the concentrations of other minerals are higher. As these results indicate, there are variations in the nutrient content depending on the location of the plant in spring and summer versus fall and winter months [11]. However, understanding the reasons for these differences is crucial. For instance, in halophyte plants, an adaptive process to the environment may be required to prevent excessive salt accumulation, maintain ionic balance within the cell, and protect the cell from oxidative stress[32]. The accumulation of carbohydrates in the cell facilitates osmotic regulation. In light of this information, it can be suggested that the Hottentot-fig plant may have accumulated carbohydrates within its structure to combat environmental stress and maintain intracellular salt balance during the spring and summer months. Moreover, the Hottentot-fig plant may have followed a strategic path to adapt to environmental changes. When all numerical results are examined, it is concluded that the Hottentot-fig species has a rich content in terms of carbohydrates, protein, and minerals such as Zn and Mn. However, the most noteworthy result in this study regarding the analysis of the nutritional profile is that the nutrient content is primarily influenced by the location and subsequently by the time of plant collection.

When examining the results of the chemical profile analysis of Hottentot-fig samples, the crucial data here are obtained from the TPC, TFC, and CTC analyses. The Hottentot-fig plant has a higher TPC ratio compared to other plants in various literature studies. However, within the scope of this study, when the TPC results of this plant in different seasons and regions A, B, C are examined, this plant species in region B, except for the summer, has yielded high results [33]. Generally, when the results of TPC for this plant species are examined, it is found that in the spring and summer months, there is a higher total phenol content compared to other months [18]. Regarding TFC content in this study, the overall flavonoid ratio is low and close to each other when compared to other analysis results [19]. The sample of Hottentot-fig obtained from the Ria Alvor region in the summer month has shown significantly higher results in terms of flavonoid content compared to others. When looking at the CTC analyses, it can be generally said that this plant contains high tannins in every region and season [34]. However, the results indicate that the highest tannin content occurs in the fall and spring. In the fall month, region B has a high tannin content, while in the spring month, the Hottentot-fig plant in the Ria Formosa region has a very high content [19]. Generally, the variations in the ratios of phenolic, flavonoid, and similar contents in plants are attributed to factors such as the location where the plant is grown, harvest time, environmental stress conditions, and extraction conditions [35]. During the spring and summer months, Hottentot-fig, like other plants, is exposed to environmental stress conditions such as drought, excessive exposure to UV radiation. To survive, they may increase phenol production in their structures as an adaptation strategy to environmental conditions in the spring and summer months. This adaptation strategy may account for the variations in TFC and CTC contents depending on the location. The analysis results of phenolic compounds have revealed the presence of phenolic acids such as salicylic, chlorogenic, caffeic, coumaric, and ferulic acids, as well as flavonoids and luteolin-7-O-glucoside compounds [24, 36]. Among these compounds, salicylic acid and luteolin-7-O-glucoside are found more in Hottentot-fig samples from regions B and C. The main reason for this is their efforts to adapt to environmental stress they are exposed to. Luteolin derivatives are produced in plants exposed to abundant sunlight and to combat soil salinity [37]. Therefore, these components are more abundant in Hottentot-fig in regions B and C. Salicylic acid provides plant immunity, while caffeic acid provides resistance to stress through its antioxidant activity and increases salt tolerance. Gentisic acid has been identified as a signaling molecule in response to defense and salinity factors in Hottentot-fig [38]. The phenolic compounds found in Hottentot-fig not only protect the plant from stress factors but also contribute to its therapeutic bioactivity [39]. Thanks to these components, this plant species possesses bioactive properties such as anti-inflammatory, antimicrobial, and antioxidant activities. The reason for Hottentot-fig's bioactive properties lies in its content of phenolic, flavonoid, and condensed tannin. Since the content of these compounds varies depending on the location and time of cultivation, it has been concluded that its bioactive properties are also location and season dependent.

The results of the analysis of the bioactivity profile of the Hottentot-fig plant include the examination of antioxidant and anti-inflammatory activities, as well as mineral content in terms of location and season. The plant samples were found to have antioxidant capacity in the analysis conducted using DPPH, ABTS, and NO methods [25]. The lower EC50 values compared to positive controls indicate that the samples have high antioxidant capacity. The Hottentot-fig's high antioxidant capacity was observed to be even higher in the analysis with ABTS radicals. The months with the most efficient antioxidant capacities were identified as spring and summer. However, it was observed that A and B regions had higher antioxidant capacities for ABTS radicals, especially during the winter season [40]. This is attributed to the production of phenolic compounds by the plant to adapt to environmental factors, as mentioned above. Additionally, the types of solvents used for plant extracts are crucial. Extracts using a water or methanol mixture will yield different antioxidant activity results depending on the solvent type. While water shows stronger activity, an extract using a methanol solvent may exhibit lower activity [41]. When examining the analysis data for the chelation properties of metal ions, copper shows a moderate chelation property compared to the positive control. However, the results for iron are relatively low. To detect the anti-inflammatory property of Hottentot-fig plant samples, it is necessary to reduce the production of NO [1, 23]. The samples of this plant, found in region B and collected during the summer months, exhibit higher anti-inflammatory activity. In conclusion, there are many factors that affect the analysis data of the bioactive profiles of the Hottentot-fig plant. From the initial stages to the final stages of the methods, all materials, chemicals, and environmental differences can impact the obtained findings. The solvents and temperatures used in plant extraction stages affect the results of these analyses [3]. However, in this study, the most important and focal point is the variation in the values of the plant's nutritional, chemical, and bioactivity profiles, which is attributed to different locations and seasonal times of cultivation for the Hottentot-fig plant. Plants grown in different locations produce specific phenolic compounds in their metabolism to adapt to the environment and protect themselves. Hottentot-fig is one of these plants. Through the production of these compounds for adaptation, it gains bioactive properties that make it potentially useful for humanity and pharmaceutical purposes.

## 4. CONCLUSION

In this study, the invasive Hottentot plant, which is found in coastal areas and poses a threat to the diversity of other plants, and its phytotherapeutic properties are examined. Although this plant species poses a crisis for plant ecology, it has the potential to be used in health and nutraceutical fields thanks to its bioactive properties such as antioxidant and antimicrobial. In the study, the techniques used to obtain the Hottentot-fig plant were examined and the results that provided the best yield were analyzed and commented on. In addition, thanks to the different bioactive components found in this plant species, it has the potential to be a resource for use in industries such as phytotherapeutics, nutraceuticals, and food. Hottentot-shank, aka *Carpobrotus edulis* (L.) N.E. Br. Considering the most efficient methodology and results for the Br plant, future studies will benefit from its therapeutic potential by turning the invasive feature of this plant into an opportunity instead of a crisis.

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