Research Article

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Phytochemical screening and antioxidant activity of *Vitex agnus-castus* L.

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Abstract: This research is dedicated to investigating the antioxidant potential and phytochemical composition of three distinct extracts derived from *Vitex agnus-castus* L. These extracts, prepared through aqueous (EXA), ethanolic (EXE), and methanolic (EXM) maceration, were chosen based on prior assessments of total polyphenol content in extracts obtained from five solvents with differing polarities: water, methanol, ethanol, acetone, and butanol. The study initiated with a comprehensive phytochemical analysis focusing on the determination of total polyphenols and flavonoids. The quantification of total polyphenols was carried out using the Folin–Ciocalteu method, while the $AlCl_3$ method was employed to assess flavonoids. In evaluating the *in vitro* antioxidant activity, we employed two well-established methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP). The preliminary tests, gauging the efficacy of solvents, demonstrated that the order of optimal solvent selection was as follows: aqueous, methanolic, ethanolic, butanolic, and acetone. Consequently, the first three solvents were chosen for the preparation of the extracts. The phytochemical analysis unveiled that EXA exhibited the highest total polyphenol content, with an impressive value of 126.84 ± 1.24 mg EAG/g extract, whereas EXE exhibited the lowest concentration of total polyphenols, measuring at 117.26 ± 0.18 mg EAG/g extract. In contrast, EXM showcased a notably high flavonoid content, registering at 33.65 ± 1.04 mg EQ/g extract, while EXA displayed a comparatively lower flavonoid content at 14.93 ± 0.14 mg EQ/g extract. When assessing antioxidant properties, EXA emerged as the most potent against both DPPH and FRAP, recording values of 78.94 ± 1.84 and 203.27 ± 0.17 µg/ml, respectively. In contrast, the ethanolic extract exhibited relatively lower antioxidant activity, with values of 204.16 ± 1.87 µg/ml for DPPH and 307.10 ± 1.15 µg/ml for FRAP.

Keywords: *Vitex agnus-castus*, polyphenols, antioxidant activity, phytochemical screening

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1 Introduction

Using plants for healing is a universal practice known for thousands of years and was the primary source of

remedies in the past. Today, this practice is referred to as "phytotherapy," which signifies medicine based on the natural active principles of plants. This designation originates from the Greek words "phuton," which translates to "plant," and "therapia," meaning "treatment." With the development of medical chemistry in the early nineteenth century, plants became the first essential source of medicine. Today, despite significant advancements in synthetic pharmaceutical chemistry and microbial fermentation, 25% of prescribed drugs in industrialized countries have a plant origin [1].

The World Health Organization considers this therapy as part of traditional medicine. It can be applied either on its own or as a complementary approach alongside other forms of medicine, whether traditional or allopathic. Caution and proper guidelines must be followed when using it. Due to its history and scope, this therapy encompasses all specialties that exist in the medical world. It is recommended for premenstrual syndrome (PMS), which includes the physical and psychological symptoms that women may experience before or during their menstruation.

Today, herbal treatments are coming to the forefront because the effectiveness of drugs such as antibiotics is declining. The use of medicinal plants in phytotherapy has garnered significant interest in biomedical research and is becoming as important as chemotherapy. This renewed interest arises from the fact that medicinal plants offer an endless source of bioactive natural substances and compounds and the need for gentler therapy with fewer side effects in the quest for improved medication [2].

Morocco has a long history in traditional medicine. It is a country with highly diverse geography and boasts a rich and varied flora, including approximately 4,200 plant species, of which 800 are recognized in traditional medicine [3].

Vitex agnus-castus L., commonly known as chaste tree or monk's pepper, has been extensively studied across diverse scientific domains owing to its historical medicinal significance. Research studies encompass a spectrum of investigations, from its traditional application in hormonal regulation, particularly in addressing menstrual irregularities and PMS, to modern pharmacological explorations elucidating its bioactive compounds, including flavonoids, iridoids, and essential oils. Studies delve into its impact on hormonal balance, notably dopamine and prolactin levels, suggesting potential effects on the endocrine system. Clinical trials and observational studies have examined its efficacy in managing PMS symptoms, irregular menstruation, breast pain, and even its role in fertility support. Additionally, investigations into its neurological effects on mood regulation and anxiety, pharmacokinetics, receptor interactions, toxicological assessments, and safety profiles contribute significantly to understanding its pharmacological actions and potential therapeutic applications. Collectively, these multidisciplinary studies bridge traditional knowledge with contemporary scientific insights, enriching our comprehension of *Vitex agnus-castus* and its plausible roles in women's health, hormonal balance, and beyond [4].

Vitex agnus castus L. is a plant that has been used for hundreds of years and may have certain efficacy. Its potential mechanisms of action are still not fully understood, but there is ongoing research partially investigating its effects.

The plant has been used for various health benefits, including the treatment of menstrual disorders, acne, and fertility issues. It has been found to contain chemicals that affect many hormones involved in the female reproductive cycle [5]. Some of the key prior works involving *Vitex agnus-castus* include studies on the effects of *Vitex agnus-castus* on hormone levels and menstrual cycle symptoms. These studies have shown that *Vitex agnus-castus* may help reduce symptoms of PMS and menopause, as well as improve fertility [6].

The aim of our work is to study the chemical composition of chaste tree seeds, characterize the different chemical groups, and assess their antioxidant activity. The interest in selecting this plant is due to the richness of our country in aromatic and medicinal plants, particularly this plant, while also highlighting that very few studies have been conducted on the seeds of the chosen plant. Therefore, this study on the chaste tree was initiated in the first instance to determine its main characteristics and more precisely, to evaluate the polyphenolic and flavonoid constituents of various extracts from (*Vitex agnus castus* L.) and assess the in vitro potential of these extracts to inhibit or protect against oxidative damage.

2 Materials and methods

2.1 Plant materials

The chaste tree seeds used in this study were purchased from an herbalist in the Beni Mellal-Azilal region. The plant material was ground into a fine powder using a blender to prepare extracts.

2.2 Extraction procedure

2.2.1 Solvent screening test

The objective of this experiment is to determine the solvents that have demonstrated the best extraction of total

polyphenols from dry plant material. To carry out this test, 50 mg of the dry plant material was placed in five test tubes, to which 1 ml of the following solvents (ethanol, methanol, acetone, distilled water, and butanol) was added, respectively. These mixtures were macerated for 24 h in the dark at room temperature. After maceration, we filtered the extracts and then proceeded with quantification using the Folin–Ciocalteu method. The measurements were taken using a spectrophotometer at a wavelength of 765 nm.

2.2.2 Preparation of extracts

A method called maceration was employed, which involves extracting the maximum amount of active compounds contained in the plant. A quantity of 50 g of the finely ground dry plant material is macerated in 1,000 mL of three solvents, namely distilled water, ethanol, and methanol, for 24 h at room temperature. The extracts obtained in this manner are vacuum-filtered, evaporated at a temperature of 40°C using a rotary evaporator to remove most of the solvent, and concentrated the extract. The remaining filtrate is subsequently placed in brown bottles.

2.3 Yield calculation

The yield is calculated using the following formula:

$$\text{Yield (\%)} = (M_{ex}/M_{th}) \times 100,$$

where M_{ex}, experimental mass (in g), is the mass of the dry extract and M_{th}, the theoretical mass (in g), is the mass of the dry plant material.

This formula expresses the yield as a percentage, indicating fraction of the theoretical mass actually obtained in the experiment.

2.4 Phytochemical screening

The obtained fractions were utilized in a preliminary phytochemical screening, a series of procedures and techniques aimed at detecting secondary metabolites present in plants. This process is qualitative in nature and relies on coloration and/or precipitation reactions to identify major chemical compound categories. Various reagents were applied for this purpose, using the analytical methods described in previous studies [7,8].

2.5 Phytochemical analysis

2.5.1 Determination of total polyphenols

The total polyphenol content of the various extracts is determined using the Folin–Ciocalteu method. The Folin–Ciocalteu reagent is a yellow-colored acid composed of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). This reagent is reduced through oxidation by phenolic compounds, resulting in a mixture of tungsten blue (W_8O_{23}) and molybdenum blue (Mo_8O_3) with a blue coloration. The intensity of this coloration is directly proportional to the levels of oxidized phenolic compounds. To do this, 2.5 mL of the Folin–Ciocalteu reagent solution (diluted ten times in distilled water) is added to 0.5 mL of the extracts in test tubes. The mixture is then vortexed to ensure thorough mixing. Next, 4 mL of 7.5% (m/v) sodium carbonate (Na_2CO_3) is added to create an alkaline environment, initiating the redox reaction. The mixture is then incubated in a water bath at 45°C for 30 min. The intensity of the blue coloration produced is measured using a spectrophotometer at a wavelength of 765 nm [9].

The study utilized a UV-visible spectrophotometer, an instrumental tool pivotal in measuring the absorption characteristics of substances across the ultraviolet and visible spectrum. The spectrophotometer employed an optical system (single or double beam) along with a light source, like deuterium and tungsten lamps, for precise wavelength illumination. Its operational range covered wavelengths from the ultraviolet (around 190 nm) to the visible range (extending up to 800 nm or beyond), ensuring comprehensive analysis. The instrument exhibited a high degree of accuracy in wavelength readings, which is vital for meticulous spectral analyses. Additionally, it offered adjustable spectral bandwidth or resolution, allowing for tailored measurement settings. Equipped with mentioned detector type, such as photodiode arrays or photomultiplier tubes, the spectrophotometer provided reliable detection and quantification of absorption values. Further details encompassed the cell holder configuration accommodating various cell sizes for sample containment during measurements. The study's spectrophotometer was complemented by sophisticated software for data acquisition and analysis, providing a user-friendly interface for control and facilitating precise interpretation of results [10].

2.5.2 Dosage of flavonoids

The quantification of flavonoids is carried out using the Dewanto colorimetric method, employing aluminum trichloride ($AlCl_3$)

and sodium hydroxide (NaOH) as reagents. $AlCl_3$ forms a yellow complex with flavonoids, and NaOH forms a pink complex that absorbs in the visible spectrum at 510 nm. In test tubes, 1 mL of each extract (1 mg/mL) and 6.4 mL of distilled water were successively introduced. Then, 0.3 mL of 5% (m/v) sodium nitrite solution ($NaNO_2$) was added. After 5 min, 0.3 mL of 10% (m/v) $AlCl_3$ was added, and the mixture was allowed to stand for 6 min. Subsequently, 2 mL of 1 M NaOH was added, and the solution was thoroughly mixed. The entire mixture was incubated in the shade at room temperature for 30 min. Absorbance was immediately measured at a wavelength of 510 nm. The total flavonoid contents in each extract were calculated by referring to the calibration curve equation, established using quercetin as the standard reference at various concentrations under the same conditions and steps of the assay [11].

2.6 Antioxidant activities

2.6.1 DPPH free radical scavenging activity

The evaluation of the antioxidant activity of various extracts against the 2,2-diphenyl-1-picrylhydrazyl (DPPH°) radical is based on the method described by Şahin et al. [12]. This method relies on measuring the ability of antioxidants to scavenge the DPPH° radical. This radical, initially violet in color, is reduced to 2,2-diphenyl-1-picrylhydrazine, which is yellow, upon accepting a hydrogen radical. The results are expressed in milligrams of quercetin equivalents per gram of extract (mg EQ/g extract).

In test tubes, 2.5 mL of different concentrations of each extract and 0.5 mL of freshly prepared methanolic DPPH solution were introduced. The mixture was vigorously vortexed and then placed in the dark at room temperature for 30 min. After vortexing, the change in the color was measured by recording the absorbance at a wavelength of 517 nm. The antioxidant activity of the extracts is expressed as IC_{50} (inhibitory concentration 50), a parameter defined as the concentration of the antioxidant that causes a 50% loss of DPPH activity.

The percentage of DPPH radical reduction (PR) is calculated using the following formula:

$$\text{Antiradical activity}(\%) = ((\text{A control} - \text{A sample}) / \text{A control}) \times 100.$$

where “A control” is the absorbance of the control and “A sample” is the absorbance of the tested samples. The measurement is performed at 517 nm, and the results are compared to a standard containing quercetin at various concentrations.

2.6.2 Ferric-reducing antioxidant power (FRAP) test

This test is based on the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The ability of our extracts to transfer electrons to the ferric iron ion was determined following the protocol established by Oyaizu et al. [13]. Concentrations of our samples and the positive control (catechin) were prepared. In each test tube, 0.2 mL of each sample, 2.5 mL of a phosphate buffer (0.2 M, pH = 6.6), and 2.5 mL of 1% potassium ferricyanide complex ($K_3Fe(CN)_6$) were added. The mixture was incubated in a water bath at 50°C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added to stop the reaction. Subsequently, 2.5 mL of each tube was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% iron chloride ($FeCl_3$).

Absorbance was measured at a wavelength of 700 nm with calibration of the spectrophotometer using the mixture without the extract. The test was conducted in triplicate, and the concentration that converts 50% of Fe^{3+} to Fe^{2+} was calculated.

2.7 Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 8 software. Data were expressed as mean ± standard deviation.

3 Results and discussion

3.1 Solvent screening test

The results of this test, expressed in milligrams equivalent gallic acid per gram of dry plant material (mg EAG/g dry matter), are shown in Table 1.

The solvent screening test conducted in this study aimed to determine the total polyphenol content of different extracts obtained from the plant material. The results, expressed in mg EAG/g dry matter, are presented in Table 1. This test assessed the effectiveness of various solvents in extracting polyphenolic compounds from the plant material and provided valuable insights into the choice of solvent for subsequent extraction processes.

The results indicate varying levels of total polyphenols in the different extracts, with significant differences among the solvents used. Notably, water extraction (50.91 mg EAG/g extract) yielded the highest concentration of polyphenols.

Table 1: Dosage of total polyphenols

Extract	Total polyphenols (mg EAG/g extract)
Ethanol	32.09 ± 1.2
Butanol	24.91 ± 0.180
Acetone	18.97 ± 0.03
Methanol	38.22 ± 1.89
Water	50.91 ± 0.13

This result underscores the efficiency of water as an extracting solvent for polyphenolic compounds, indicating its potential as an excellent choice for the extraction of these valuable phytochemicals.

Methanol extraction (38.22 mg EAG/g extract) also demonstrated a high polyphenol content, making it a suitable option for extracting polyphenols from the plant material. Ethanol extraction (32.09 mg EAG/g extract) provided a moderate but notable level of polyphenols, indicating its effectiveness in capturing these compounds.

Butanol (24.91 mg EAG/g extract) and acetone (18.97 mg EAG/g extract) extractions resulted in relatively lower levels of polyphenols compared to the other solvents. These solvents may be less efficient at extracting polyphenolic compounds from the plant material.

In summary, the solvent screening test highlights the critical role of the choice of solvent in extracting polyphenolic compounds from the plant material. Water and methanol emerge as the most effective solvents for extracting polyphenols, with water being particularly efficient in this regard. These results have practical implications for the design of extraction processes for polyphenols, emphasizing the importance of selecting an appropriate solvent to maximize the yield of these valuable compounds.

3.2 Yield calculation

The extraction yield is the ratio of the quantity of substances extracted by the extracting action of a solvent to the quantity of plant material. It depends on several parameters, such as the solvent used, temperature, extraction time, and the composition of the sample. The yields of the aqueous, ethanolic, and butanolic extracts of chaste tree seeds are provided in Table 2.

The result of the extraction yields for the chaste tree (*Vitex agnus castus* L.) extracts show that the ethanolic extract has a yield of approximately 6.62%, which is the highest yield, followed by the aqueous extract at 5.8%, and finally, the methanolic extract has the lowest yield at 4.9%. Extraction yield depends on several factors that can influence the extraction performance, such as the solubilization capacity of compounds, temperature, and extraction time.

Table 2: Yield of different extracts of *Vitex agnus castus* L.

Plant extracts	Color	Yield (%)
Aqueous extract	Verdate brown	5.8
Methanolic extract	Dark green	4.9
Ethanolic extract	Dark green	6.62

3.3 Phytochemical screening

The identification of various classes of secondary metabolites present in plants provides insight into their pharmacological activities. We conducted phytochemical tests on the aqueous, ethanolic, and butanolic extracts of chaste tree seeds. These tests are related to the intensity of precipitation and coloration, which are proportional to the quantity of the sought-after substance. The results of the phytochemical screening of chaste trees are presented in Table 3.

Table 3 offers a detailed insight into the phytochemical composition of *Vitex agnus-castus* L., presenting a diverse array of compounds within the plant. Alkaloids, indicated by positive reactions with Mayer and Dragendorff reagents, suggest their presence, aligning with their known medicinal significance.

The substantial presence of polyphenols underscores the plant's antioxidant potential, which is crucial for its possible health benefits. Within the flavonoid category, while flavones and flavonols exhibit varied intensities,

Table 3: Phytochemical characterization of different chaste tree seed extracts

Compounds		Plant
Alkaloids	Mayer reagent	+
	Dragendorff reagent	++
Polyphenols		+++
Flavonoids	Flavonols	–
	Flavones	++
	Flavonones	–
Tannins	Catechins	–
	Gallic and ellagic	–
Terpenoids		+++
Sterols and triterpenes		+++

The presence of chemical compounds is indicated as follows: (+++) significant, (++) moderate, (+) slight, and (–) absent.

the absence of flavonones suggests their limited presence. Tannins, particularly catechins, gallic, and ellagic types, appear to be relatively low or absent.

Conversely, terpenoids and sterols/triterpenes demonstrate considerable abundance, indicating their potential pharmacological significance. This comprehensive profile of compounds in *Vitex agnus-castus* underscores their complex chemical composition, hinting at their potential therapeutic and medicinal applications that deserve further exploration and research.

3.4 Phytochemical analysis

3.4.1 Determination of total polyphenols

The quantification of total polyphenols was performed through extrapolation based on a calibration curve generated using a standard (gallic acid) at various concentrations, following the same conditions as the extracts. The results are expressed in milligrams of gallic acid equivalent per gram of dry plant material (mg EAG/g extract), utilizing the linear regression equation of the gallic acid calibration curve.

The total polyphenol content, expressed in milligrams equivalent gallic acid per gram of extract (mg EAG/g extract), is presented in Table 4.

Table 4 provides insight into the total polyphenol content of the aqueous extract, methanolic extract, and ethanolic extract of *Vitex agnus-castus*, expressed in milligrams equivalent gallic acid per gram of extract (mg EAG/g extract). Polyphenols are renowned for their antioxidant properties and potential health benefits. This analysis allows us to understand the polyphenolic composition of these extracts and their potential as sources of antioxidants.

The aqueous extract exhibited the highest total polyphenol content among the three extracts, with a value of 126.84 mg EAG/g extract. This result indicates a substantial concentration of polyphenolic compounds within the aqueous extract.

The methanolic extract also displayed a considerable total polyphenol content, measured at 121.64 mg EAG/g extract. This value underscores the presence of polyphenolic compounds within the methanolic extract, which contributes to its antioxidant potential.

Similarly, the ethanolic extract showed a substantial total polyphenol content, with a value of 117.26 mg EAG/g extract. This result indicates that the ethanolic extract also contains a significant amount of polyphenolic compounds, reinforcing its potential as an antioxidant source.

In summary, the total polyphenol content analysis reveals that all three extracts of *Vitex agnus-castus* are rich in polyphenolic compounds. The aqueous extract exhibits the highest concentration, followed closely by the methanolic and ethanolic extracts. These polyphenols are essential contributors to the antioxidant potential of these extracts.

The variations in total polyphenol content highlight the diverse composition of bioactive compounds present in *Vitex agnus-castus*, depending on the extraction method employed. These differences play a role in the varying levels of antioxidant potential observed in previous assays.

3.4.2 Dosage of flavonoids

The concentration of flavonoids in chaste tree (*Vitex agnus castus* L.) extracts is determined using a spectrophotometric method with aluminum chloride. The content of flavonoids is expressed in milligrams of quercetin equivalent per gram of extract (mg EQ/g extract).

The total polyphenol content, expressed in milligrams equivalent quercetin per gram of extract (mg EQ/g extract), is presented in Table 5.

Table 5 presents the contents of flavonoids in the aqueous extract, methanolic extract, and ethanolic extract of *Vitex agnus-castus*, expressed in milligrams equivalent quercetin per gram of extract (mg EQ/g extract). Flavonoids are a subgroup of polyphenolic compounds known for their antioxidant properties and various health benefits. Comparing the flavonoid content of these extracts provides insights into their potential antioxidant capabilities.

The aqueous extract exhibited a flavonoid content of 14.93 mg EQ/g extract. While this value indicates the presence of flavonoids in the extract, it is essential to recognize that this is a lower concentration compared to the

Table 4: Total polyphenol assay results of *Vitex agnus castus* L.

Plant extracts	Total polyphenol content (mg EAG/g extract)
Aqueous extract	126.84 ± 1.24
Methanolic extract	121.64 ± 2.10
Ethanolic extract	117.26 ± 0.18

Table 5: Contents of flavonoids of *Vitex agnus castus* L.

Plant extracts	Contents of flavonoids (mg EQ/g extract)
Aqueous extract	14.93 ± 0.14
Methanolic extract	33.65 ± 1.04
Ethanolic extract	25.775 ± 0.84

other extracts. Flavonoids are key contributors to antioxidant activity, and their presence suggests potential free radical-scavenging capabilities within the aqueous extract.

In contrast, the methanolic extract contained a notably higher flavonoid content of 33.65 mg EQ/g extract. This finding suggests that the methanolic extract is rich in flavonoids, which are likely responsible for a significant portion of its antioxidant potential.

The ethanolic extract also contained a substantial flavonoid content, measured at 25.775 mg EQ/g extract. While slightly lower than the methanolic extract, this value indicates a considerable presence of flavonoids in the ethanolic extract, emphasizing its potential as an antioxidant source.

In summary, the flavonoid content analysis demonstrates that both the methanolic and ethanolic extracts of *Vitex agnus-castus* contain a substantial amount of flavonoids, which are well-documented for their antioxidant properties. The aqueous extract, while showing a lower flavonoid content, still exhibits the presence of these important compounds.

The variation in flavonoid content among the extracts underscores the diverse composition of bioactive compounds within *Vitex agnus-castus*, depending on the extraction method. These differences contribute to the varying levels of antioxidant potential observed in previous assays.

3.5 Antioxidant activities

3.5.1 DPPH free radical scavenging activity

In this study, antioxidant activity is defined based on the percentage of scavenging (or inhibition) of the free radical DPPH. Since there is no absolute measure of the antioxidant capacity of a compound, the results are expressed relative to quercetin as a reference antioxidant and are presented in Table 6.

The results of the antioxidant activity evaluation for the methanolic extract, ethanolic extract, and aqueous extract of *Vitex agnus-castus*, in comparison to the reference compound quercetin [14], provide valuable insights into the potential use of these extracts as sources of natural antioxidants.

Quercetin, a well-known flavonoid with potent antioxidant properties, served as the reference standard in this study. It exhibited a remarkably low IC_{50} value of 5.49 µg/ml, highlighting its strong antioxidant activity. This result validates the sensitivity and reliability of the DPPH assay used in the study and serves as a benchmark for assessing the extracts' antioxidant potential.

In contrast, the methanolic extract, with an IC_{50} value of 207.89 µg/ml, and the ethanolic extract, with an IC_{50} value of 204.16 µg/ml, displayed moderately higher IC_{50} values when compared to quercetin. These values suggest that both the methanolic and ethanolic extracts possess moderate antioxidant activity. While not as potent as quercetin, their ability to neutralize free radicals indicates that they can contribute to reducing oxidative stress and related damage.

Table 6: Antioxidant activity of extracts of *Vitex agnus castus* L. using DPPH

Plant extracts	DPPH IC_{50} (µg/ml)
Aqueous extract	78.94 ± 1.84
Methanolic extract	207.89 ± 0.67
Ethanolic extract	204.16 ± 1.87
Quercetin	5.49 ± 0.02

The aqueous extract of *Vitex agnus-castus* demonstrated the strongest antioxidant activity among the tested extracts, with an IC_{50} value of 78.94 µg/ml. Although its IC_{50} value is higher than that of quercetin, it is important to note that natural extracts often contain a complex mixture of compounds, each with its own antioxidant properties. This diversity of antioxidants can have a cumulative effect, potentially enhancing their overall capacity to combat oxidative stress and protect cells from damage.

In summary, the methanolic and ethanolic extracts, while not as potent as quercetin, display moderate antioxidant activity, which is promising for their potential use as natural antioxidants. The aqueous extract, while exhibiting the strongest antioxidant activity among the tested extracts, also shows potential as a source of antioxidants. These findings underscore the diversity of antioxidant compounds present in *Vitex agnus-castus* through different extraction methods.

3.5.2 FRAP test

The evaluation of the antioxidant activity of chaste tree seed extracts using the FRAP method is based on the reduction of ferric ions Fe^{3+} to ferrous ions Fe^{2+}. The presence of ferrous ions can be assessed by measuring and monitoring the increase in absorbance of the reaction medium at 700 nm. Catechin is used as the reference antioxidant. Table 7 presents the values of IC_{50} concentrations (the concentration equivalent to an absorbance of 0.5) obtained for catechin and for each chaste tree extract.

Catechin, a well-known and widely studied flavonoid, serves as the reference compound in this study. It displayed a notably low FRAP EC_{50} value of 19.54 µg/ml, indicating its

strong antioxidant activity. This result underscores the reliability of the FRAP assay in assessing antioxidant potential and sets a high benchmark for the extracts' performance.

In comparison, the methanolic extract exhibited an FRAP EC_{50} value of 307.10 µg/ml, indicating relatively lower antioxidant activity when compared to catechin [14]. Similarly, the ethanolic extract displayed an FRAP EC_{50} value of 211.98 µg/ml, suggesting moderate antioxidant potential. While both extracts show higher EC_{50} values compared to catechin, these values are still within a range that implies antioxidant capacity.

The aqueous extract, with an FRAP EC_{50} value of 203.27 µg/ml, also demonstrated moderate antioxidant activity. While its EC_{50} value is higher than that of catechin, it is important to consider that natural extracts often contain a mixture of antioxidants, which, when combined, can contribute to a cumulative antioxidant effect.

These findings highlight that the aqueous extract, methanolic extract, and ethanolic extract all possess antioxidant potential, albeit at different levels when compared to the reference compound catechin. These variations in the antioxidant activity may be attributed to differences in the composition of bioactive compounds within each extract, as well as the efficiency of the extraction methods employed.

In conclusion, the results of the FRAP assay suggest that while the methanolic, ethanolic, and aqueous extracts of *Vitex agnus-castus* may not match the exceptional antioxidant activity of catechin, they still exhibit moderate to low antioxidant potential. This is an encouraging finding, as it implies that these extracts can contribute to reducing oxidative stress and its associated health risks.

The results reported by Hajdú et al. [15], l'extrait d'acétate d'éthyle des fruits de *Vitex agnus-castus* a été examiné. Cet extrait contient des composants flavonoïdes, en particulier la casticine, la vitexine et l'orientine. Les flavonoïdes sont une classe de composés polyphénoliques naturels connus pour leurs puissants effets antioxydants. Ces composés ont la capacité de neutraliser les radicaux libres nocifs et de réduire le stress oxydatif dans l'organisme. La présence de ces flavonoïdes dans l'extrait d'acétate d'éthyle suggère que cette fraction de *Vitex agnus-castus* possède un potentiel antioxydant.

Table 7: Antioxidant activity of extracts of *Vitex agnus castus* L. using FRAP

Plant extracts	FRAP EC_{50} (µg/ml)
Aqueous extract	203.27 ± 0.17
Methanolic extract	307.10 ± 1.15
Ethanolic extract	211.98 ± 0.74
Catechin	19.54 ± 0.25

According to Sağlam et al. [16], a porté sur les extraits d'éthanol, de *n*-hexane et d'eau des feuilles et des fruits de la plante. Ces extraits contiennent des flavonoïdes et des tanins, tous deux reconnus pour leurs propriétés antioxydantes. Les flavonoïdes sont connus pour leur capacité à piéger les radicaux libres, tandis que les tanins peuvent chélater efficacement les ions métalliques qui contribuent aux dommages oxydatifs. La diversité des composés antioxydants présents dans les différentes parties de la plante souligne le potentiel du *Vitex agnus-castus* à fournir des effets antioxydants complets.

Selon Sarikurkcu et al. [17], divers extraits, dont l'huile essentielle, l'eau, l'hexane, le dichlorométhane, l'acétate d'éthyle et les extraits de méthanol des fruits de *Vitex agnus-castus*, ont été analysés pour leur teneur en composés phénoliques et en flavonoïdes. Les composés phénoliques, en particulier les acides phénoliques et les flavonoïdes, jouent un rôle clé dans la capacité antioxydante de nombreuses plantes. La présence de composés phénoliques et flavonoïdes dans ces extraits indique leur potentiel d'atténuation du stress oxydatif.

L'étude de Mesaik et al. [18], s'est concentrée sur l'extrait méthanolique des feuilles et a révélé la présence de flavonoïdes et de composés phénoliques totaux. Cette découverte suggère que les feuilles de *Vitex agnus-castus* contiennent également des constituants antioxydants précieux, élargissant ainsi les applications potentielles de la plante au-delà de ses fruits.

The findings from these studies emphasize the varied chemical composition of Vitex agnus-castus and its promising role as a natural antioxidant source. These antioxidants are crucial in shielding against oxidative damage, a factor implicated in various health issues, including aging, inflammatory diseases, and chronic conditions.

4 Conclusion

Vitex agnus-castus, commonly known as chaste tree, is recognized as a medicinal and aromatic plant owing to its abundant secondary metabolites. It serves as a significant reservoir of diverse bioactive molecules renowned for their antioxidant, antimicrobial, and pharmacological properties. Our study pursued a dual objective: initially, to identify the most effective solvent for extraction and determine the yields of fixed extracts from *Vitex agnus-castus* seeds using maceration; subsequently, to conduct a qualitative and quantitative analysis of secondary metabolites and assess their *in vitro* antioxidant potential within these fixed extracts. Phytochemical examinations of the extracts derived from

Vitex agnus-castus seeds unveiled substantial levels of polyphenols, flavonoids, and terpenoids. Evaluation of the antioxidant prowess of these extracts through the ferric reduction assay (FRAP) accentuated their ability to reduce ferric iron, notably showcasing the aqueous extract with the highest antioxidant potency. Assessment of the seed extracts' antioxidant capabilities via the DPPH test across varying concentrations revealed robust antioxidant activity across most extracts, with the aqueous extract displaying the lowest IC50 value of 78.94 ± 1.84 compared to others. These findings underscore the inherent antioxidant capacity of the studied plant as established through the DPPH and FRAP assessments.

Future investigations in subsequent years should focus on exploring the antidiabetic, anti-inflammatory, and antimicrobial attributes of the bioactive compounds sourced from this plant. Moreover, efforts to determine their therapeutic dosage and elucidate their cellular mechanisms of action are imperative. Such advancements hold promise for the development of pharmaceutical products showing significant therapeutic potential. Additionally, the presence of phenolic compounds in our plant imparts diverse biological properties, prompting the need for employing complementary methods, like HPLC-MS, to comprehensively describe and identify specific metabolites.

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