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Comparative Analysis of Phenolic and Flavonoid content, Antioxidant, Antibacterial Activities, and Functional Groups of Chemicals from *Hypericum perforatum* L., and *Papaver Rhoeas* L. Flower Extracts

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ABSTRACT

In this study, the authors aimed to compare the phytochemical compounds (polyphenols and flavonoids), antioxidant activity, functional groups present in the compounds (FTIR), and anti-microbial effects, in the aqueous and methanol extracts obtained from flowers of commercial *Hypericum perforatum* and native *Papaver Rhoeas* from Morocco. *P. Rhoeas* L was collected from El Lhaj Kaddour near Meknes, while *H. perforatum* L was bought in a dried state from a Moroccan herbalist in the same city. Total polyphenols were evaluated using the Folin-Ciocalteu reagent, respectively. The antioxidant activity was assessed via DPPH and anti-microbial effects were tested against six bacteria (Gram– and Gram+). The aqueous and methanol extracts of *P. Rhoeas* had the highest TPC value (23.67 ± 0.94 mg GAE/g; 15.86 ± 0.65 mg GAE/g) compared to *H. perforatum* (15.26 ± 1.30 mg GAE/g; 5.50 ± 1.13 GAE/g). The aqueous extract of *Papaver Rhoeas* exhibited the highest TFC at 14.36 ± 0.49 mg QE/g, while the methanol extract of *Hypericum perforatum* had the highest 10.65 ± 0.49 mg QE/g in TFC. In contrast, the methanol and aqueous extracts of *H. perforatum* showed significant zones of inhibition against Staphylococcus aureus (9.5 ± 0.5 mm and 10.17 ± 0.29 mm) and Staphylococcus epidermidis (8.33 ± 0.58 mm and 9.33 ± 0.58 mm) respectively, with a minimum inhibitory concentration was estimated at 10 µL/ml. The FTIR analysis demonstrated that the extracts of both plants are rich in bioactive molecules with potential biological activities and a pharmaceutical industry perspective. Consequently, these *Papaver Rhoeas* and *Hypericum perforatum* extracts exhibit antioxidant and antibacterial activities.

Keywords: *Hypercium perforatum*; *Papaver rhoeas*; phenolics; flavonoids; antioxidant activity; antibacterial; FTIR spectroscopic analysis.

INTRODUCTION

The use of medicinal herbs has a long historical lineage, playing a pivotal role in the evolution of medical practices. Ancestral wisdom has significantly contributed to the development of numerous contemporary medications, highlighting their antibacterial and antioxidant attributes, as well as the presence of polyphenolic and flavonoid compounds (Süntar, 2020; Zrouri et al., 2023). Ongoing scientific investigations continue to explore the potential of these therapeutic herbs (Wang et al., 2021). This combination of traditional knowledge and scientific advancements enhances the understanding of medicinal herbs and their relevance in modern healthcare (Pimm et al., 2014). It is noteworthy that approximately ten percent of vascular plants possess therapeutic properties, with estimates suggesting a range of 350,000 to over 500,000 species exhibiting such attributes. Throughout history, plants have been utilized for the management of various ailments, and this practice continues to persist in the present day (Malik, 2017). Plants are living chemical factories that biosynthesize a vast array of secondary metabolites (Sarrocco, 2016). These metabolites are, in fact, are the building blocks of many pharmaceutical drugs available commercially, and herbal remedies crafted from medicinal plants (Boukhatem & Setzer, 2020). The biological activity of medicinal plants is closely linked to the chemical composition of these species. Chemical compounds, including flavonoids, polyphenols, and alkaloids, are the principal molecules that support the anti-bacterial and antioxidant activities of extracts from plants. As a member of the Hypericaceae family, *Hypericum perforatum* L, a fragrant perennial herb known for its rhizomatous growth pattern and can reach a maximum height of approximately 1 meter. The plant produces vibrant yellow flowers that are organized in a wide, branching cymose inflorescence at the apex of its stem (Dehgan, 2022). The *H. perforatum* extracts contain numerous phenolic compounds, specifically flavonoids and phenolic acids, indicating that they possess significant important antioxidant properties and antibacterial (Tusevski et al., 2017). *P. Rhoeas* L. is a yearly and perennial plant that belong to the Papaveraceae family. This species is generally appreciated as an attractive plant due to its beautiful cup-shaped flowers that come in different colors, often with bicolor or semi-double combinations. Chemical analysis has revealed the presence of various alkaloids, such as oripavine and thebaine, within this plant. Additionally, this species is used for medicinal purposes, including the treatment of gastric ulcers, sleep disorders and coughs, and making it a significant medicinal plant (Zhou et al., 2018). In recent years, the growing resistance of bacteria to the currently available antimicrobial medicines have become a global health issue among scientists and doctors. Among the potential sources of these agents, plants are considered the most promising. The antimicrobial properties of natural products were explored, and in this case, the excesses of the plants were discovered in an active area of the search (Mohamed et al., 2019). These efforts are being made to discover effective solutions in combating microbial resistance and to contribute to new treatment strategies. Some medicinal plant extracts have shown the ability to inhibit drug-resistant microorganisms (Ahmed et al., 2020; Maione et al., 2016). For example, NK Mohammed studied the phytochemical composition of methanolic extracts of *Anethum Graveolens* and Plantago Major using FTIR spectroscopic analysis. Subsequently, the anti-bacterial activity of these extracts was tested against pathogenic microorganism strains, of pathogenic microorganisms including *Escherichia coli, Proteus mirabilias, Klebsiella pneumoniae and Staphylococcus aureus*. The study findings revealed that both plant extracts exhibited significant inhibition capacity against all tested microorganisms (Mohammed, 2021).

The conducted study aimed to compare the chemical composition and biological properties of wild *P. Rhoeas* and commercially available *Hypericum perforatum*. Additionally, the impact of different extraction solvents on the biological functions and the chemical composition of both plants was assessed. Specifically, variations in the levels of total polyphenols and flavonoids in aqueous and methanol extracts derived from the flowers of *P. Rhoeas* and *H. perforatum* were examined. FTIR Spectroscopic analysis was used to identify the functional groups present in the compounds of the extracts in the studied plants (to detect functional groups of compounds). Subsequently, their effects on antioxidant and antibacterial activities were explored, with antibacterial assessments conducted against six bacteria, for both Gram-positive and negative strains.

MATERIALS AND METHODS

Plant material

In this study, two plant species, i.e. *Papaver Rhoeas* L., and *Hypericum perforatum* were used to analyze the phytochemicals and biological properties. *P. Rhoeas* was collected from El Lhaj Kaddour, Meknes (33°49'47" N, 5°25'17" W) during the flowering season in March-May 2021. In contrast, *Hypericum perforatum* L was purchased in a dried state from a Moroccan herbalist who specialized in the sale of imported plants of foreign origin. The purchased plant originated from France. Both plants were transported to the Laboratory of Environment and Plant Protection (EPP) at the National School of Agriculture of Meknes. The identification of both plants was performed by Professor Belmelha; a botanist at the Department of Environment and Plant Protection. *Hypericum perforatum* L was required in dried form, while flowers of *P. Rhoeas* were air-dried for 20 days, and then ground for future experiments.

Extraction process

To prepare aqueous extracts, 10 g of powdered flowers from each plant were added and macerated the mixture for 24 hours at room temperature with magnetic stirring in 100 ml of distilled water. The aqueous phase of the macerated powder was then filtered through Whatman N1 filter paper and stored in dark bottles at 4 °C (Fercha & Bousselsela, 2014). The methanol extracts of both species were prepared according to the procedure outlined by Ben Saadi & Guemmouda, (2017). In the considered case, 500 ml of methanol was macerated with 50 g of flower powder for 24 hours at room temperature with magnetic stirring. Subsequently, the aqueous phase of the macerate was screened with Whatman N1 filter paper. When the extraction process was complete, the organic extract was concentrated in a rotary evaporator (Bushi) at 40 degrees Celsius under vacuum (Kasdi et al., 2017).

Total phenolic compounds (TPC)

The total polyphenols were determined using the Folin-Ciocalteu reagent in spectrophotometry, employing a colorimetric approach. This reagent consists of a combination of phosphotungstic acid and phosphomolybdic acid. To each glass tube, 400 µl of each previously prepared extract was added, along with 1600 µl of a 7.5% sodium carbonate solution and 2 ml of a ten-fold diluted Folin-Ciocalteu reagent. After shaking the tubes and allowing them to stand in the dark for half an hour, the absorbance at 765 nm was measured. Three readings of each were conducted. Using the same working conditions, a calibration curve was generated in parallel using gallic acid. The results were expressed as mg GAE/g.

Total flavonoid content (TFC)

To quantify the overall flavonoid content, 2 ml from each extract or standard, prepared using methanol, was combined with 2 ml of 2% $AlCl_3$ solution, also prepared in methanol. After one hour of incubation in a light-restricted environment, the absorbance of the sample was quantified at a wavelength of 430 nm. Each reading was replicated three times. The calibration process used quercetin under identical experimental circumstances as the sample. The findings were shown in milligrams equivalent of Quercetin per gram of dry matter (mg QE/g).

Antioxidant activity

To evaluate the antioxidant activity of prepared extracts of *P. rhoeas*, and *H. perforatum*, the approach proposed by Bouyahya et al., (2017) was adopted. However, the original method was slightly modified to accommodate the conducted experiments. In the considered case, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was altered. Using a syringe and some water, a vapor that can be used to make a variety of products, including cosmetics, was created. Plant extracts at various concentrations (30–480 µg/ml) were added to 3.2 ml of a 0.11 mM methanolic DPPH solution. The absorbance was measured at 517 nm against a methanol blank and the DPPH solution after 30 minutes of incubation in the dark.

The radical-scavenging activity of DPPH was calculated as follows:

$$\%(AA) = \frac{\text{Abs 517 control} - \text{Abs 517 sample}}{\text{Abs 517 control}} \times 100 \quad (1)$$

The extract concentration that inhibits the DPPH radical by 50% (IC50) is determined from the graph of inhibition percentage vs extract concentration using the exponential equation and ascorbic acid was used as a positive control.

Antibacterial activity

The assessment of the antimicrobial efficacy of extracts derived from the studied plants was initially conducted using the disc diffusion technique, as described by Oubihi et al., (2020). The procedure involved introducing a microbial suspension with an optical density of CFU/mL onto Muller Hinton agar medium by flooding Petri dishes. The ethanolic and methanol extracts were dissolved in DMSO (Dimethylsulfoxide). The choice of DMSO was supported by its widespread use among researchers, notably Gachkar et al., (2007) who demonstrated that DMSO does not possess significant antibacterial efficacy. In the considered case, 6 mm diameter sterile discs made of Whatman paper were saturated with 15 µl of the extract and placed on the agar surface at the center of each dish. Synthetic antibiotic discs containing amoxicillin (25 µg) and penicillin (5 µg) were utilized as positive controls. The incubation process was conducted at a temperature of 37 °C for a duration of 24 hours. The findings were quantified by assessing the transparent region surrounding the disk. The efficacy of the extract was evaluated using a panel of six microbiological strains, comprising two Gram+ bacteria (*Staphylococcus epidermidis* and *Staphylococcus aureus*) and four Gram- bacteria (*Klebsiella pneumoniae, Enterobacter cloacae, Escherichia coli,* and *Acinetobacter baumannii*).

The minimum inhibitory (MIC) and bactericidal (MBC) concentrations

The minimal inhibitory concentrations (MIC) and bactericidal concentrations (MBC) were determined using the agar dilution method (Jaber et al., 2021). The extracts were emulsified by using an agar solution with a 0.2% concentration. Various dilutions were generated in the agar solution, specifically at concentrations of 2 µL mL^{-1}, 3.3 µL mL^{-1}, 5 µL mL^{-1}, 10 µL mL^{-1}, 20 µL mL^{-1}, 40 µLmL^{-1}, and 100 µL mL^{-1}. In a laboratory setting, test tubes having 13.5 milliliters of solid medium were used. These tubes were sterilized in an autoclave at a temperature of 121 degrees Celsius. Subsequently, 1.5 milliliters of each dilution were introduced into the test tubes, resulting in final concentrations of 10 microliters per milliliter, 4 microliters per milliliter, 2 microliters per milliliter, 1 microliter per milliliter, 0.5 microliters per milliliter, 0.33 microliters per milliliter, and 0.2 microliters per milliliter. The contents of each tube were promptly transferred into a sterile Petri dish. In addition, cookies were produced by including the culture medium and a 0.2% agar solution. The process of seeding involves streaking with a calibrated platinum loop to ensure the collection of a consistent volume of inoculum. The latter was presented as a 24-hour culture broth. The incubation process was conducted for a duration of 24 hours at a temperature of 37 °C. To reduce experimental error, the test was conducted in triplicate.

FTIR analysis

Many people consider the Fourier transform infrared spectrophotometer (FTIR) as an exceedingly useful tool for identifying and characterizing functional groups found in a variety of substances. The present study proposed a methodology for characterizing several types of samples, including liquids, solutions, pastes, powders, films, fibers, and gases, as outlined by Nandiyanto et al., (2019). The primary focus of this study was to desiccate materials, specifically powders and methanolic extracts. In the considered case, a small quantity of each powdered sample and methanolic extract was analyzed in the mid-infrared radiation range, specifically at the wavelengths of 450 cm^{-1} and 4000 cm^{-1}. This analysis was conducted using the "Perkin-Elmer LS 55" spectrophotometer, which was integrated with the "PerkinElmer Spectrum TM 10" software, enabling the presentation of the data in the form of spectra. The analysis was conducted in triplicate to ensure the confirmation of the spectrum.

Statistical analysis

The Kolmogorov-Smirnov test was used to determine if all measured variables, including antioxidant activity, total phenolic compounds, and total flavonoid content, followed a normal distribution. Then, the researched parameters were compared with ANOVA and Tukey tests (tree replicates for each parameter). To test the correspondence between the inhibitory effect and treated bacteria, Correspondence Analysis was used. Extracts (n=2) were considered as factors, while the tested microorganisms were considered as dependent variable. Statistical analysis was realized with Graph Pad Prism 5 and the significance level was determined at 95% ($p<0.05$).

RESULTS AND DISCUSSION

Total flavonoid and phenolic contents

The results presented in Table 1 summarize the measurements of total phenolic and flavonoid contents in various extracts of *H. perforatum*, and *P. rhoeas*. These findings indicate that the overall levels of flavonoids and phenolic compounds varied depending on the plant and the type of extract used. Notably, the aqueous extract of *P. Rhoeas* exhibited the highest total phenolic content (TPC), with an estimated value of 23.67 ± 0.94 mg GAE/g, surpassing the aqueous extract of *H. perforatum*, which had a TPC of 15.26 ± 1.30 mg GAE/g. Similarly, the methanol extract of *P. Rhoeas* had the highest TPC value, measuring at 15.86 ± 0.65 mg GAE/g, in contrast to the methanol extract of *H. perforatum*, which had a TPC of 5.50 ± 1.13 mg GAE/g. When it comes to total flavonoid content (TFC), the aqueous extract of *P. Rhoeas* also displayed the highest value, with an estimated 14.36 ± 0.49 mg QE/g, while the aqueous extract of *H. perforatum* had a TFC of 8.25 ± 1.08 mg QE/g. In contrast, the methanol extract of *H. perforatum* showed the highest TFC value, with a measurement of 10.65 ± 0.49 mg QE/g, surpassing the methanol extract of *P. rhoeas*, which had a TFC of 8.19 ± 1.55 mg QE/g.

This study provides new insights into the chemical composition of indigenous *P. Rhoeas* from Morocco and commercially available *H. perforatum*. The aqueous and methanol extracts of *P. Rhoeas* exhibited the greatest total phenolic content (TPC) value. However, the methanol extracts showed lower total flavonoid content (TFC) values compared to *H. perforatum*. Necip & Mesut, (2019) conducted a study on the bioactive compounds found in different fractions of *H. perforatum* extracted using different solvents. The authors utilized various extracts, including methanol, aqueous, and HPL aqueous extracts. The TPC in the water extracts of *H. perforatum* was determined to be 32.27 ± 3.6 µg GAE.mg^{-1} extract, while in methanol extracts, the TPC value was 30.3 ± 3.9 µg GAE.mg^{-1} extract. Kirca & Arslan, (2008) determined that the methanol extracts of the plant contained an approximate amount of TPC of 30.30 ± 3.9 µg GAE.mg^{-1} extract. The levels of phenolic compounds in the samples collected from Greece varied between 125 ± 1, and 228 ± 2 mg of gallic acid per gram of dry extract (Gioti et al., 2009).

It was proposed that two primary reasons, either the deterioration of quality in commercialized samples or the geographical origin of the selected plant in comparison to those of the bibliography, regulate the difference in TPC quantities between the obtained results and those of the mentioned researchers. According to numerous studies conducted recently, commercialized plants have a lower chemical composition, including polyphenols, than their wild counterparts (Tewari et al., 2017). For example, as reported by Sánchez-Velázquez et al., (2021), wild blackberries (Rubus spp.) have an estimated 127 EAG of TPC per mL, while commercial varieties have 79 mg EAG per mL. Likewise, Chimshirova et al., (2019) discovered that the TPC concentrations in fresh extracts of the leaves and flowers of wild *Hypericum perforatum* ranged from 11.0 to 56.69 mg GAE g^{-1} dw, while the extracts from commercial plants maintained for nine months had TPC concentrations between 9.33 and 50.98 mg GAE g^{-1} dw. Regarding the influence of geographic location, Makarova et al., (2021) reported a TPC content in *Hypericum perforatum* estimated at 371 mg GAE/g in the samples obtained from Poland, whereas Chimshirova et al., (2019) reported a TPC content in the samples obtained from Bulgarian territory estimated at 10.20 ± 1.23 to 53.84 ± 4.04 mg GAE.g^{-1}. Conversely, Marsoul et al., (2020) assessed the amount of polyphenols present in methanolic extracts (maceration and soxhlet) made from *P. Rhoeas* flowers that were gathered in the Fez (Morocco) region.

Table 1. Total polyphenol contents and total flavonoid contents in the *P. rhoeas*, and *H. perforatum* extracts

Type of extract	Species	TPC mg GAE/g	TFC mg QE/g
Water extract	*P. Rhoeas*	23.67 ± 0.94^{a**}	14.36 ± 0.49^{a**}
	H. perforatum	15.26 ± 1.30^{a*}	8.25 ± 1.08^{b*}
Methanol extract	*P. Rhoeas*	15.86 ± 0.65^{b**}	8.19 ± 1.55^{b*}
	H. perforatum	5.50 ± 1.13^{b*}	10.65 ± 0.49^{a**}

Note: Tukey's multiple range test comparing between the extracts of the same plant (a>b; $p < 0.05$) and the same extract of both plants (**>*; $p < 0.05$).

The soxhlet extracts and the maceration were found to have total phenolic contents (TPC) of 165.4 ± 3.84 and 95.4 ± 2.42 mg GAE/g, respectively. Hmamou et al., (2022) assessed the antimicrobial properties and chemical compounds (TPC and TFC) in extracts (hydro-ethanolic maceration with 70% ethanol and 30% distilled water) from *P. Rhoeas* flowers, stems, roots, and leaves that were collected from the Taounate region (North of Morocco). The leaf extract had the highest TPC value in the results, measuring 24.24 ± 4.96 mg GAE/g of extract. The flower extract came in second with 22.10 ± 2.22 mg GAE/g of extract. These outcomes closely resemble those seen in the methanolic extract of the studied samples. This is hypothesized to be caused by the shared geographic origin of the samples (Morocco) in both this study and the literature, which is now being discussed by Hmamou et al., (2023).

The obtained findings showed varying results in terms of total flavonoid levels depending on the extracts and the plants species. The highest TFC in methanol extract was found in Hypericum perforatum. In the aerial root of *Hypericum perforatum*, Sobhani Najafabadi et al., (2019) reported values of total flavonoid content ranging between 20 and 80 mg/g dry weight, which are higher than the measured quantity of TFC. Yousuf et al., (2012) examined phytochemicals in a methanolic extract of the *Hypericum perforatum* leaves taken from India. They calculated the flavonoid content to be 17.10 ± 0.02 mg (RE)/g of material. Yaman et al., (2022) examined the phytochemical composition of the callus, *in vitro* plantlet, leaves, flowers, and stems of *H. perforatum* in comparison to wild samples. On the basis of the obtained data, the projected TFC values were 26.6 mg QE/g in the methanol extract from flowers, 46.1 mg QE/g in the leaves, and 102.4 mg QE/g in the stem of the wild *H. perforatum*. The value of TFC in *in vitro* plantlets was 18.5 mg QE/g extract, while in callus, it was 9.3. In the samples of *Hypericum perforatum* recorded from Poland, Makarova et al., (2021) found an estimated 160 mg CAE/g of TFC. Sobhani Najafabadi et al., (2019) found that the samples from the western region of North Macedonia had estimated values ranging from 61.64 to 125.35 mg CE/g, whereas the samples from Turkey were found to have 70.61±0.51 µg QEs/mg according to Ersoy et al., (2020). The differences in the TFC values between the studied commercial results and those found in the literature for native and planted varieties of *Hypericum perforatum* highlight the significance of sample storage duration and geographic origin. As a matter of fact, Sobhani Najafabadi et al., (2019) showed that the amounts of TFC varied according to storage times and exposure to light. When samples were stored for one week as opposed to four weeks, and when they were exposed to blue light as opposed to normal and dark settings, the amount of total flavonoids (TFC) was lower (Sobhani Najafabadi et al., 2019). Similar to this, it is hypothesized that inadequate storage conditions and exposure to light in herbalists are the causes of the reduced TFC levels in the studied samples. Likewise, the source of the samples may have an impact on the amount of TFC.

Conversely, when compared to methanol extract, the maximum TFC was found in the aqueous extracts of *P. rhoeas*. Presently, Marsoul et al., (2020) assessed the total flavonoids in methanol extracts from *P. Rhoeas* flowers and found that, in the maceration and soxhlet procedures, the values were respectively 8.67 ± 0.024 mg QE/g and 21.7 ± 2.05 mg QE/g. These outcomes are similar to what was found in this study. This was implied to be controlled by the same source as both the studied samples and the published samples. The amount of TFC in three samples of *P. Rhoeas* from diverse origins counting Taounate, Fez, and Sefrou in the Fez-Meknes region was examined by Hmamou et al., (2023). The results showed that the TFC levels were 4.47 ± 0.30, 4.44 ± 0.286, and 4.72 ± 0.346 mg QE/g of dry extract, respectively. It was not statistically different from the other outcomes.

Assessment of the antioxidant activity

The results distinctly revealed pronounced antioxidant efficacy. Table 2 displays the antioxidant activity results in extracts of *Hypericum perforatum* and *P. rhoeas*. The methanol extract of *Hypericum perforatum* had the highest IC50 value 77.815 ± 0.03 µg/mL, in comparison with the methanol extract of *P. Rhoeas* 26.60 ± 4.80 µg/mL. The aqueous extract of *P. Rhoeas* had the highest IC50 value, 54.50 ± 3.45 µg/mL, in comparison with the aqueous extract of *Hypericum perforatum* 15.403 ± 0.01µg/ml.

According to Mohtar et al., (2020), the IC50 values represent the lowest antioxidant concentration required to achieve 50% reactivity. The ability of a plant to scavenge DPPH radicals is frequently used as an indicator of its potential

Table 2. Comparison of DPPH free radical scavenging activity between methanolic and aqueous extracts of *P. rhoeas*, and *H. perforatum*

Type of extract	DPPH-IC_{50} (µg/mL)	
	P. rhoeas	*H. perforatum*
Water extract	54.50 ± 3.45^{a**}	15.403 ± 0.01^{b*}
Methanol extract	26.60 ± 4.80^{b*}	77.815 ± 0.03^{a**}

Note: Tukey's multiple range test comparing between the extracts of the same plant (a>b; $p < 0.05$) and the same extract of both plants (**>*; $p < 0.05$).

antioxidant capacity (Sadaf et al., 2021). In their study, Chimshirova et al., (2019) assessed the antioxidant capacity of ethanolic extracts from the leaves and flowers of both commercial and wild varieties of *Hypericum perforatum* L. Consequently, for wild plants, the IC50 value ranged from 2.50% to 11.15%, and for commercial plants, it varied from 3.82% to 26.68%. An extended storage period of studied extracts resulted in a decrease in the IC50 value for both wild and marketed plants. The value of the IC50 in commercial *Hypericum perforatum* was 26.68% after 2 minutes of storage, and it dropped to 3.82% after 120 minutes. Conversely, the DPPH-IC50 of the aqueous extracts of the studied commercial *Hypericum perforatum* was 15.403 ± 0.01 µg/mL, which is extremely similar to the values reported by Chimshirova et al., (2019). On the other hand, the studied methanol extracts, which contained 77.815 ± 0.03 µg/mL of *Hypericum perforatum* flowers, were far better than those reported by Chimshirova et al., (2019). This was true for both commercialized and wild varieties. Rey-Méndez et al., (2022) assessed the antioxidant activity of *Hypericum perforatum* L. flower, stem, and leaf extracts in order to produce gold nanoparticles. The enhanced DPPH radical scavenging capacity of the nanoparticles after synthesis was revealed with IC50 values of 0.11 0.05 for flowers, 0.23 0.002 for leaves, and 0.11 0.002 mg/m. This is in contrast to the corresponding ethanolic plant extracts, which had IC50 values of 0.71 0.04 for flowers, 0.71 0.03 for leaves, and 0.69 0.004 mg/mL for stems. The abundance of phenols and flavonoids in the studied samples is hypothesized to support their increased capacity to scavenge free radicals (Gonçalves et al., 2018). Chua et al., (2013) also showed that phenolic chemicals have a major role in the antioxidant activity of plant materials. In a similar vein, the studies conducted by Lourenço et al., (2019) and Yu et al., (2021)have proposed that the primary sources of natural antioxidants in plants used in food are phenolic compounds, such those present in *Hypericum perforatum* L. In vitro tests were conducted by Hmamou et al., (2022) to examine

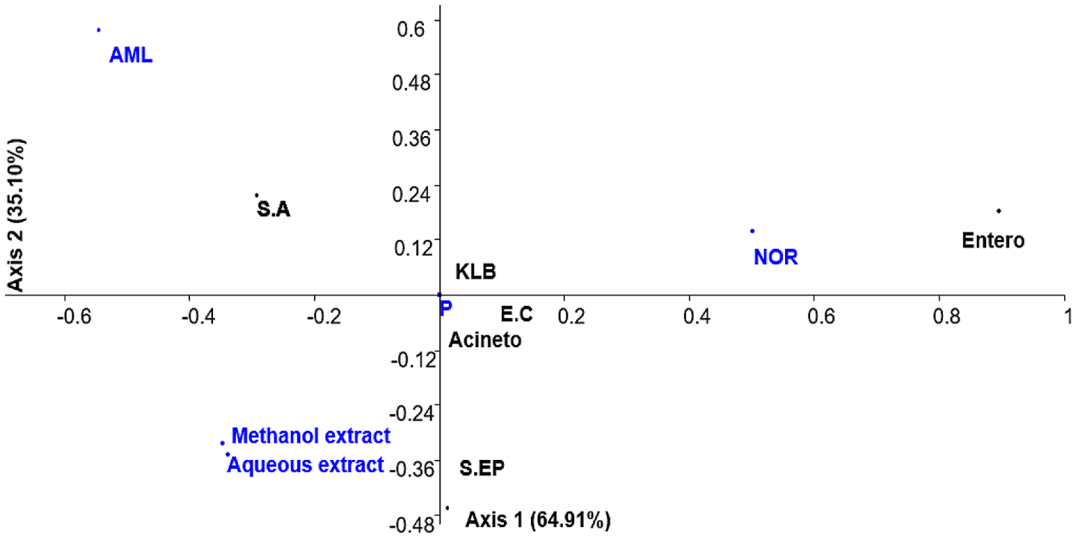

Figure 1. Correspondance analysis of inhibitory effects of extracts and synthetic antibiotics, and treated microorganisms (P: Penicilline 5 µg; NOR: Norfloxacine 5 µg; AMP: Amoxicillin 10 µg; S.A: *Staphylococcus aureus*; S.EP: *Staphylococcus epidermidis*; KLB: *Klebsiella pneumonia*; E.C: *Escherichia coli*; Acineto: *Acinetobacter baumannii*; Entero: *Enterobacter cloacae*)

the antioxidant capacity of several organ extracts from *P. rhoeas*, the the data obtained indicated that the extracts from leaves had the an estimated value of DPPH scavenging activity of 0.50 ± 0.007 mg/mL. Comparably, the IC50 of extract from flowers was estimated at 0.52 ± 0.005 mg/mL. Hmamou et al., (2023) investigated the biological activities of *Papaver Rhoeas* L. extracts from dissimilar geographical areas of Morocco. The DPPH IC50 values of *P. Rhoeas* extracts were estimated between 0.28 and 0.38 mg/mL. Marsoul et al., (2020) examined the antioxidant activity of soxhlet and maceration extracts made from *P. Rhoeas* flowers collected in the central Moroccan region of Fez. When compared to maceration extract (IC50 = 4.97 mg/ml), soxhlet extract (IC50 = 3.81 mg/ml) was shown to have the highest and most significant antioxidant potential. Presently, methanolic extracts of four *P. Rhoeas* sections taken in Central Morocco were examined for their antioxidant activities. The lowest recorded value of 0.50 ± 0.007 mg/mL was found in leaves, but the flowers had an estimated IC50 of 0.52 ± 0.005 mg/mL. The greatest IC50 values were found in the roots and stems, at 2.12 ± 0.044 mg/mL and 1.56 ± 0.027 mg/mL, respectively. The conducted analysis of the bibliography and authors' own data shows that the antioxidant activity varies depending on the region of origin, the solvents used, and the portion of *P. Rhoeas* that is extracted. The impact of chemical content, specifically flavonoids and polyphenols, on the antioxidant activity of plant extracts was also proposed in authors' instance (Castro-López et al., 2017).

Antibacterial activity

The results concerning the antibacterial properties of flower extracts from *P. rhoeas*, and *H. perforatum*, along with their comparison to synthetic antibiotics, are outlined in Table 3. The extracts derived from both *P. rhoeas*, and *H. perforatum* exhibited varying outcomes when tested against the target microorganisms (Figure 1). Notably, the extracts of *P. Rhoeas* displayed no antimicrobial effects against either Gram-negative or Gram-positive bacteria. In the case of *H. perforatum*, the inhibitory effects of the extracts varied significantly based on the type of extract and the specific microorganisms being tested. Both the methanol and aqueous extracts from *H. perforatum* demonstrated substantial inhibitory activity against *S. aureus*, and *S. epidermidis*. The aqueous extract from *H. perforatum* exhibited an estimated inhibition zone of 10.17 ± 0.29 mm against *S. aureus*, while the methanol extract showed a slightly smaller zone of 9.5 ± 0.50 mm. Similarly, against *S. epidermidis*, the aqueous extract yielded an estimated inhibition zone of 9.33 ± 0.58 mm, compared to 8.33 ± 0.58 mm for the methanol extract. However, when it came to *K. pneumoniae* (KLB), *E. coli* (E.C), *A. baumannii* (Acineto), and *E. cloacae* (Entero), all the tested extracts of *H. perforatum* exhibited no inhibitory effects. In contrast, synthetic antibiotics demonstrated inhibitory effects against only three microorganisms, with variable inhibition zone diameters. Norfloxacine displayed inhibitory effects against *S. aureus* (SA), *E. cloacae*, and *S. epidermidis* (S.EP) with estimated diameters of 21 mm,

Table 3. Antibacterial effects of the methanol and aqueous extracts from *H. perforatum* and *P. Rhoeas* and their comparison with synthetic antibiotics against tested microorganisms

Species	Microorganisms	Extracts		Synthetic antibiotics		
		Methanolic	Aqueous	Norfloxacine	Penicilline	Amoxicillin
H. perforatum	S. A	9.5 ± 0.5	10.17 ± 0.29	21	0	13
	S. EP	8.33 ± 0.58	9.33 ± 0.58	15	0	0
	KLB	0.00 ± 0.00	0.00 ± 0.00	0	0	0
	ACINETO	0.00 ± 0.00	0.00 ± 0.00	0	0	0
	E. C	0.00 ± 0.00	0.00 ± 0.00	0	0	0
	ENTERO	0.00 ± 0.00	0.00 ± 0.00	16	0	0
P. rhoeas	S. A	0.00 ± 0.00	0.00 ± 0.00	21	0	13
	S. EP	0.00 ± 0.00	0.00 ± 0.00	15	0	0
	KLB	0.00 ± 0.00	0.00 ± 0.00	0	0	0
	ACINETO	0.00 ± 0.00	0.00 ± 0.00	0	0	0
	E. C	0.00 ± 0.00	0.00 ± 0.00	0	0	0
	ENTERO	0.00 ± 0.00	0.00 ± 0.00	16	0	0

Table 4. MIC and MBC of extracts against tested bacteria

Species	Extract HPF methanol		Extract HPF Aq	
	MI (µL/mL)	CMB (µL/mL)	CMI (µL/mL)	CMB (µL/mL)
S. aureus	10	10	10	10
S. epidermidis	10	10	10	10

16 mm, and 15 mm, respectively. Amoxicillin inhibited the growth of only Staphylococcus aureus (S.A) with an estimated diameter of 13 mm. Conversely, Penicilline exhibited no inhibitory effects against any of the tested bacteria.

Table 4 displays the results of the minimal inhibitory and minimal bactericidal concentrations of methanol and aqueous extracts. The minimal inhibitory concentration of aqueous and methanol extracts is similar between Gram-positive bacteria and was estimated at 10 µL/mL against *S. aureus*, and *S. epidermidis*. Similarly, the minimal bactericidal concentration was equal for Gram-positive bacteria and was estimated at 10 µL/mL against *S. aureus*, and *S. epidermidis*.

Numerous studies have explored the inhibitory effects of various extracts derived from *H. perforatum*. For example, Nazlı et al., (2019) investigated the Antibiofilm and antimicrobial activity of Polyurethane and *H. perforatum* extract (PHPE). *C. albicans, E. coli,* and *S. aureus* were the three clinical pathogens against which the antimicrobial efficacy of *H. perforatum* extract was tested. The *H. perforatum* extract had the strongest antibacterial activity against the *S. aureus* strain. The findings of the antibiofilm research showed that the S. aureus biofilm production also suppressed *H. perforatum* by 56.85%. Comparing the polyurethane material and *H. perforatum* extract (PHPE) group to the control group, the amount of S. aureus biofilm was reduced by 92.85%. An investigation using scanning electron microscopy (SEM) demonstrated the decrease in S. aureus following the integration of *H. perforatum*. In another study, (Ghodrati et al., (2021) examined the antibacterial properties of the methanolic extract of *Hypericum perforatum* against microorganisms that are present in food. The diameter of the halo of non-growth of bacteria against the extract made of methanol varied from 28.15 ± 0.45 to 33.9 ± 0.60 mm. In *Pseudomonas aeruginosa*, the inhibition zone was 27.12 ± 0.53 mm, in *Escherichia coli* was 20.13 ± 0.59 mm, and in *Staphylococcus aureus* was 28.15 ± 0.60 mm. The methanolic extract had dose-dependent ($P<0.05$) antimicrobial properties. The results showed that *Escherichia coli* (0.50 and 1.00, respectively) and *Staphylococcus aureus* (0.0010 and 0.0019, respectively) had the lowest and highest MIC and MBC. Currently, Gul et al., (2023) compared the antimicrobial activities of the *Hypericum perforatum* L. species grown In Türkiye. The highest inhibition zones of the methanolic extract were recorded against *S. aureus* ATCC 6538 (10 mm). The lowest MIC values were 200 µg mL^{-1} against *S. aureus* ATCC 6538, *C. krusei* ATCC 6258, *E. faecalis* ATCC 51299, *P. aeruginosa* ATCC 27853. In a different investigation, Rahnavard, (2015), examined the antibacterial activity of extracts from cultivated *H. perforatum*, particularly against methicillin-resistant *S. aureus*. The tested bacteria exhibited varying effects at different dilutions, with the purest dilution having the most significant impact and a 0.125 mg dilution resulting in the lowest effect. The inhibitory properties of extracts from *H. perforatum* in both the conducted research and existing literature are attributed to the presence of chemical compounds known for their antibacterial properties. For example, hypericin from the methanol extracts of *H. perforatum* demonstrated effective inhibitory activity against Methicillin-Resistant *S. aureus* (Rahnavard, 2015). Consequently, the extracts from this plant species are utilized in the synthesis of green products like nanoparticles (silver and gold) with notable biological properties (Alahmad et al., 2021, 2022; Rey-Méndez et al., 2022). For instance, in a study by Alahmad et al., (2022), an aqueous extract from *H. perforatum* was used to synthesize silver nanoparticles, which were then tested for their antibacterial activity against clinical and foodborne pathogens. While *E. coli* strains were resistant to the tested AgNPs, the synthesized particles exhibited antibacterial activity against both Gram-positive and Gram-negative bacterial strains, forming inhibitory zones ranging from 13 to 32 mm with MIC values of 6.25–12.5 µg/mL. The tested AgNPs at concentrations equal to or greater than half of the MIC significantly reduced the specific growth rate of *S. aureus*.

In the considered case, the antibacterial activity is suggested to be governed by the chemical composition of the extracts. Flavonoids and polyphenols have demonstrated their capacity to inhibit various microorganisms including those with antibiotic resistance such as *S. aureus* (Lopes et al., 2017; Pagliarulo et al., 2016). Polyphenols and flavonoids target the cell wall of bacteria, including membrane receptors, lipid membranes, and ion channels causing irreversible damage, that lead to inhibition of bacteria (Chen et al., 2017). Equally, polyphenols and flavonoids impact the intracellular constituents of bacteria, including proteins, nucleotides, enzymes, and metabolites, disrupting the functioning of bacteria cells (Chen et al., 2017; Lobiuc et al., 2023).

FTIR analysis

Figures 2 and 3 illustrate the FTIR-ATR spectra of powdered *P. Rhoeas* (PR), as well as their respective methanol extracts. The observed FTIR peaks are attributed to the bending and stretching vibrations of the primary structural groups present in the studied samples. Notably, the infrared spectra in the 4000–500 cm^{-1} range reveal distinctive absorption patterns and intensities that differentiate HP from PR, aligning with the findings of the phytochemical screening (Table 1) and antioxidant profile (Table 2). These peaks are primarily characterized by a broad band associated with the O–H stretching of phenolic hydroxyl groups, intermolecular hydrogen bonds, and the C–H stretching of terminal alkynes (Gitea et al., 2020). The results of the antioxidant content analysis indicate that PR samples exhibit more pronounced peaks than HP, with higher total phenolic content (TPC) and total flavonoid content (TFC) in the ethanolic and methanolic extracts of PR compared to those from HP (Table 1). A smaller peak in the 2950–2900 cm^{-1} range corresponds to the C–H stretching vibration of methyl groups, with both samples exhibiting this peak at 2018 and 2019 cm^{-1}, albeit at varying intensities. The PR sample displays the strongest peak, while the HP sample shows the weakest intensity. Additionally, a shoulder peak at 2850 cm^{-1} is linked to the C-H symmetric stretching mode of methyl groups (Gitea et al., 2020).

The results of the antioxidant content analysis show that the most significant PR sample has more pronounced peaks than HP, with TPC and TFC being greater in the ethanolic and methanolic extracts of PR than in the extracts from HP (Table 1). A smaller peak in the 2950–2900 cm^{-1} range is attributed to the C–H stretch vibration of methyl groups. Both samples exhibited this peak (2018 and 2019 cm^{-1}), but it was found at varying intensities; in fact, the PR sample had the strongest peak, while the HP sample showed the least intense of peak. The C-H symmetric stretching mode of methyl groups is associated with a shoulder peak at 2850 cm^{-1} (Gitea et al., 2020). Both samples had the same intensities in this zone. The asymmetric bending vibration C=O of amino acids, flavonoids, and lipids is responsible for the band seen at 1735 cm^{-1}, and it is associated with the stretching mode of carbonyl moiety (Ibrahim et al., 2018). In addition to the N–H asymmetric stretching of amino acids, the large band seen in the 1700–1500 cm^{-1} range, particularly at 1602 and 1608 cm^{-1}, is attributed to the C=O and C=C stretching vibration of flavonoids (Ibrahim et al., 2018). According to Ashokkumar & Ramaswamy, (2014), the HP sample exhibits absorption at around 1448 cm^{-1} and 1409 cm^{-1} in HP and PR powder, respectively. This is most likely caused by C–H stretching. According to Lehto et al., (2018), the weak bands at 1279 cm^{-1} (HP) and 1258 cm^{-1} (PR) correlate to the aromatic rings' C–O–C stretching vibration. The fingerprint region, which spans from 1200 to 500 cm^{-1}, is distinguished by a strong peak, with shoulders located at 1016 cm^{-1} for the HP sample and 1047 cm^{-1} for the PR sample. C-H aromatic out of the plane is indicated by weak absorption at 950–500 cm^{-1} (Alahmad et al., 2022). Rey-Méndez et al., (2022) employed Fourier transform infrared spectroscopy (FTIR) to find potential structural modifications and functional groups related to the reduction of the gold ions from the gold synthetized nanoparticles of *Hypericum perforatum* leaf extracts in order to make additional comparisons. The primary variations in the data are found in the 1800–1600 cm^{-1} region, where two bands (1774.6 and 1651.7 cm^{-1}) that were part of the *Hypericum perforatum* spectrum have vanished. Similar to this, Hmamou et al., (2023) showed the antibacterial ability of bioactive compounds from *P. Rhoeas* to suppress microbes with other possible medicinal applications using polarized microscopy and Fourier transform infrared spectroscopy (FTIR). In a similar vein, Lehto et al., (2018) employed Fourier transform infrared (FTIR) to analyze the spectra of their biosynthesized green synthesis nanoparticles of silver as well as the

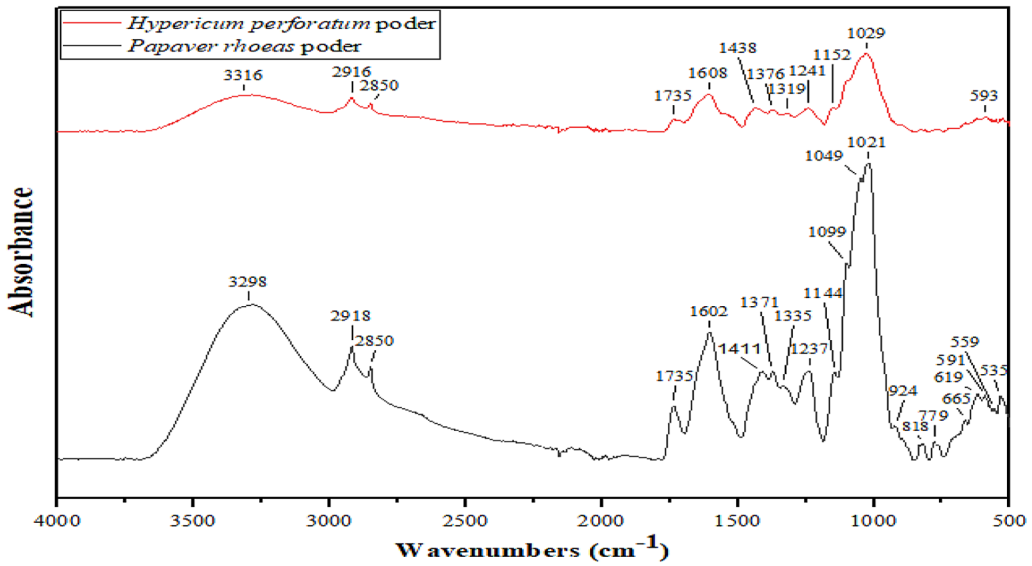

Figure 2. FTIR-ATR spectrum of powder of both of the studied plants (*Hypericum perforatum* (HP) and *P. Rhoeas* (PR))

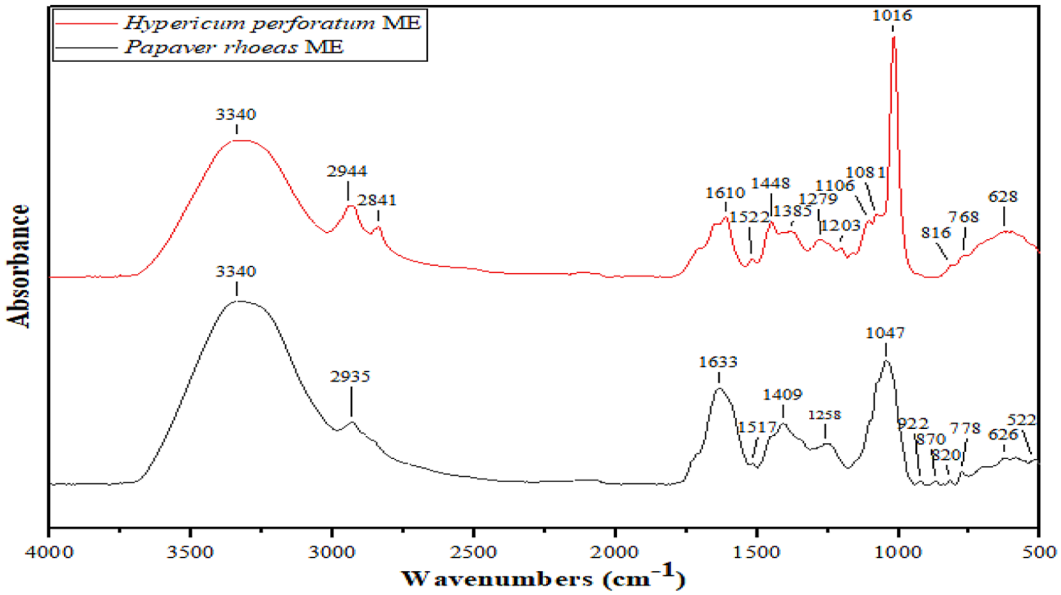

Figure 3. FTIR-ATR spectrum of methanolic extract of both of the studied plants (*Hypericum perforatum* (HP) and *P. Rhoeas* (PR))

aqueous extract from *P. Rhoeas* leaves. The extract from *P. Rhoeas* showed peaks at 1013, 1233, 1599, 1733, 2113, 2847, 2918, and 3280 cm^{-1}, according to the data. In contrast, the peaks at 536, 797, 1151, 1986, 2019, 2322, 2653, and 3736 cm^{-1} were shown by silver nanoparticles that were biosynthesized using Green Synthesis Technique. Additionally, the alcohol or phenol -OH stretch was identified as the cause of the broad peak at 3280 cm^{-1} (Renuka et al., 2020). C–H (alkaline) stress peaks were identified as significant peaks at 2653, 2847, and 2918 cm^{-1} (Sharma et al 2019). The phosphate and isocyanate groups may also be associated with the signal at 2322 cm^{-1}. Tensile vibration is represented by the peaks (-C≡N or -C≡C-) at 2113 and 2109 cm^{-1} (Lehto et al., 2018).

CONCLUSIONS

This study compared the phytochemical components (polyphenols and flavonoids), antioxidant activity, FTIR, and anti-microbial properties of extracts obtained from flowers of commercially available *H. perforatum,* and native *P. Rhoeas* from Morocco. The data obtained indicated that

the levels of total polyphenols and flavonoids varied among plants, depending on the type of extract used. The aqueous extracts of both *H. perforatum*, and *P. Rhoeas* had a higher total phenolic content compared to the methanolic extract. In addition, the TPC value was markedly higher in the methanol and aqueous extracts of *P. Rhoeas* compared to the extracts of *H. perforatum*. Furthermore, the aqueous extracts of *P. Rhoeas* exhibited a higher Total flavonoid concentration compared to *H. perforatum*. The TFC value was markedly higher in the water extract of *P. Rhoeas* compared to the methanol extract. Conversely, the methanol extract of *H. perforatum* exhibited the highest TFC value when compared to the aqueous extract. The aqueous extract of *P. Rhoeas* exhibited the strongest antioxidant ability, whereas the methanol extract of *H. perforatum* showed the highest recorded DPPH value. The extracts of both *H. perforatum* exhibited notable antibacterial activity against *S. aureus*, and *S. epidermidis*, as evidenced by substantial inhibition zones and the lowest minimal inhibitory concentration. Conversely, all examined bacteria exhibited resistance to both methanol and aqueous extracts of *P. rhoeas*. The FTIR analysis revealed that the extracts of both plants have a high concentration of bioactive compounds with the potential to exhibit significant biological activities, making them valuable from a pharmaceutical business standpoint. Nevertheless, further research is required to compare the phytochemical components of indigenous and commercialized variations. This comparison is recommended to promote the extensive utilization of available resources and the preservation of wild plants, especially if their compositions are comparable.

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