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Comparative assessment of total phenolics content and in vitro antioxidant capacity variations of leaf extracts of *Origanum grossii* and *Thymus pallidus*

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Abstract: The objective of this research was to assess the efficacy of extracts derived from *Origanum grossii* and *Thymus pallidus* leaves, focusing on their inherent phenolic compounds with varying antioxidant processes. The extracts were obtained through the utilization of a Soxhlet apparatus for hot extraction. Several parameters including overall phenolic and flavonoid content, total antioxidant activity (TAC), ferric reduction capacity (FRAP), DPPH scavenging capacity, and ABTS capability were investigated. The total phenolic content ranged from 292.91±1.51 to 3.804±0.22 mg/g DW in oregano samples and from 284.3±1.78 to 3.435±0.23 mg/g DW in thyme fractions, employing gallic acid equivalents as the measurement unit. The sequence of extraction effectiveness, ranked from highest to lowest, was methanolic extracts > aqueous extracts > ethyl acetate extracts for both, oregano and thyme. Furthermore, the methanolic extracts displayed the greatest reducing and antiradical power, along with the highest total antioxidant capacities, for both plant species. These remarkable antioxidant properties of the extracts demonstrated a strong correlation with the levels of total phenols (TP) and total flavonoids (TF). Oregano and thyme leaves showcased promising antioxidant capacities, underscoring their potential as abundant sources of phenolic compounds with diverse antioxidant characteristics, thereby indicating their viability as natural preservatives.

Keywords: *Moroccan plants, Antioxidant activity, Total phenolic, Oregano, Thyme*

1. Introduction

The process of oxidation is a fundamental and natural occurrence in the human body, playing a crucial role in numerous physiological processes (El Ouadi *et al.*, 2015; Pizzino *et al.*, 2017; Elmsellem, *et al.*, 2019). It gives rise to highly reactive oxygen species called free radicals, which have the potential to inflict significant damage to cells (Pizzino *et al.*, 2017). When there is an excessive accumulation of these free radicals and an imbalance between their production and the body's antioxidant defense processes, it leads to a condition known as oxidative stress (Percário *et al.*, 2020). This state of imbalance has been strongly associated with various chronic illnesses, including cancer, Parkinson's disease, Alzheimer's disease, as well as cardiovascular and other neurological disorders (Vatner *et al.*, 2020; Farihi *et al.*, 2023). These free radicals can induce oxidative damage to biomolecules such as proteins, lipids, and DNA, disrupting their normal functions and contributing to the progression of these diseases (Mooli *et al.*, 2022).

Our bodies possess a highly intricate and intricate mechanism of antioxidants designed to counteract the detrimental impact of free radicals (Hendrix *et al.*, 2020). This system comprises internal enzymes and compounds that collaborate to eliminate and neutralize free radicals, safeguarding cells and tissues from oxidative harm (Shiau *et al.*, 2022). Antioxidants function by offering hydrogen atoms or electrons to free radicals, effectively stabilizing them and halting further damage (Lourenço *et al.*, 2019). The antioxidant defense mechanism plays a critical role in maintaining the delicate balance between oxidation and antioxidant activity, thereby ensuring the proper functioning of our cells and tissues (Mendonça *et al.*, 2022). Given these facts, there has been a notable surge in interest in studying natural antioxidants derived from medicinal plants (Amalich *et al.*, 2016). Thyme and oregano, both belonging to the Lamiaceae family, are renowned for their remarkable health benefits. These aromatic herbs have been used for centuries in traditional medicine and culinary practices. Rich in bioactive compounds, they offer a wide array of therapeutic properties (Lee *et al.*, 2020).

Thyme genus is a perennial herb that contains potent antioxidants such as thymol and carvacrol (Zejli et *al.*, 2023). These compounds have been shown to exhibit antimicrobial, anti-inflammatory, and antitumor activities (Salehi *et al.*, 2018). Thyme is also known for its expectorant properties, making it beneficial for respiratory health. It has been traditionally used to alleviate coughs, congestion, and sore throat (Palmieri *et al.*, 2020). Oregano is another member of the Lamiaceae family that possesses notable health benefits (Boskovic *et al.*, 2019). It contains various bioactive compounds including rosmarinic acid, thymol, and carvacrol, which contribute to its antioxidant, antimicrobial, and anti-inflammatory properties (Lu *et al.*, 2018; Rodriguez-Garcia *et al.*, 2016; Vallverdú-Queralt *et al.*, 2014). Oregano has been traditionally used to support digestion, relieve gastrointestinal discomfort, and combat infections (Bouyahya & Jamal, 2016). *Thyme and Oregano* have undergone thorough research to explore their potential in preventing and treating chronic illnesses. Studies have demonstrated their ability to combat bacteria, fungi, and parasites due to their antimicrobial effects (Giannenas *et al.*, 2018; Szilvaśsy *et al.*, 2013). Furthermore, their antioxidant properties aid in neutralizing detrimental free radicals within the body, consequently reducing oxidative stress and promoting general health (Budini *et al.*, 1980). Research on the potential of oregano and thyme for preventing and treating chronic illnesses has been extensive (El-Gharbaoui *et al.*, 2017). Studies have shown that their antibacterial activities make them effective against bacteria, fungus, and parasites. Additionally, by neutralizing harmful free radicals within the body, their antioxidant qualities help to lower oxidative stress and advance general wellness. By delving deeper into the antioxidant potential of thyme and oregano, we can gain valuable insights into the specific bioactive compounds responsible for their antioxidant properties (Ortega-Ramirez *et al.*, 2015; Stahl-Biskup & Saez, 2002).

The primary objective of this study is to determine the polyphenol and flavonoid content of *Thymus pallidus* and *Origanum grossii* extracts. In addition to assessing the polyphenol and flavonoid content, we will comprehensively evaluate the antioxidant activity of thyme and oregano extracts using a diverse array of well-established methodologies. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay will be employed to measure the direct antioxidant activity of the samples by assessing their ability to neutralize free radicals. The FRAP (Ferric Reducing Antioxidant Power) assay will quantify the samples' capacity to reduce ferric forms of iron, while the ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic)) assay will measure antioxidant activity through an electron transfer mechanism. Furthermore, the TAC (Total Antioxidant Capacity) assay will be used to evaluate the overall capacity of the samples to neutralize various reactive oxygen species (Živković *et al.*, 2019).

By exploring the antioxidant activity of thyme and oregano and investigating potential correlations between their polyphenol and flavonoid content, this study aims to unearth novel sources of natural

antioxidants with significant health benefits. The findings from this research may have far-reaching implications, particularly within the food, pharmaceutical, and cosmetics industries, where there is a growing demand for natural and functional ingredients.

2. Methodology

2.1 Sourcing and preparation of extracts

In June 2018, leaves from *Origanum grossi* and *Thymus pallidus* were carefully collected from Ribat El-kheir, Sefrou. To preserve their integrity, the leaves were shade dried for a duration of two weeks. Subsequently, they were finely ground into a powder form. The extraction process employed was the hot preparation method using a Soxhlet apparatus. For each extraction, 5 grams of oregano or thyme leaf powder was mixed with 50 mL of solvent, which included distilled water, methanol, and ethyl acetate. The extraction process was carried out sequentially. Following extraction, the resulting fractions were stored in a refrigerated environment within an airtight bottle, ensuring protection against light and oxygen until they were ready for use. This careful preservation maintained the quality and potency of the extracted compounds for further analysis and experimentation.

2.2 HPLC-MS analysis

The study's methodology, influenced by a previous comparative study conducted by (Zefzoufi *et al.*, 2021), employed Ultra High-Performance Liquid Chromatography (HPLC) with Diode Array Detection and Electrospray Ionization Mass Spectrometry (UHPLC-DAD-ESI/MS) using the Dionex Ultimate 3000 system from the USA. A Kinetex C18 reversed-phase column facilitated chromatographic separation. The elution gradient involved a formic acid aqueous solution (solvent A) and methanol (solvent B), with a specified program. UV-Vis spectral measurements were taken in the 200–400 nm range, and chromatographic profiles were recorded at 280 nm. The triple quadrupole (TSQ) Endura mass spectrometer (Thermo Fisher Scientific, CA, USA) was utilized in negative mode (H-ESI) with specific settings. The method involved full scan MS acquisition mode, comparing mass data with the NIST MD Search 2.3 library and an internal library of standards for structure assignment.

2.3. The total phenolic content (TPC)

In a manner reminiscent of the methodology outlined by Slinkard and Singleton in 1977, the extract underwent a specific dilution (0.5 mL), followed by the addition of 2.5 mL of a 10% (v/v) Folin-Ciocalteu reagent and 2 mL of a 7% sodium carbonate solution. Afterward, the resulting combination was allowed to undergo incubation at ambient temperature in complete absence of light for a period of two hours. Subsequent to the incubation period, the absorbance at a wavelength of 760 nm was recorded. A calibration graph was generated utilizing gallic acid as the benchmark standard. The total phenolic content was quantified and presented as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW), thereby providing a standardized measure of phenolic content relative to gallic acid (Slinkard & Singleton, 1977).

2.4 The total flavonoids content (TFC)

This method, as described by Djeridane *et al.*, 2006, in their work from 2006, was used to determine TFC. In this process, a diluted sample (1.5 mL) was combined with a 2% aluminum chloride (AlCl3) solution in the same volume (1.5 mL), and the mixture was let to sit for 30 minutes at room temperature. Following the incubation, the mixture's absorbance was measured at a wavelength of 430 nm in comparison to a control. Rutin was used as the reference standard to create a calibration curve that was

used to determine the TFC's concentration, which was then represented as milligrams of Rutin equivalents per gram of dry weight (mg RE/g DW) (Djeridane *et al*., 2006).

2.5 The DPPH antiradical scavenging ability

The antiradical activity of the extracts was evaluated using a commonly employed method. In this assay, different concentrations of the extract or a standard antioxidant were mixed with a methanolic solution containing DPPH (0.004%), along with methanol. The mixture was vigorously shaken and kept in darkness at room temperature. After a 30-minute incubation period, the absorbance was measured at 517 nm. The percentage of inhibition was calculated using the following formula:

DPPH scavenging capacity (%) = ((A0 - A1) / A0) * 100

Where A0 represents the absorbance of the control at 30 minutes, and A1 represents the absorbance of the sample at 30 minutes. The concentration required to inhibit 50% of the initial free radicals (IC50) was determined by plotting the inhibition percentage on a graph (Barbouchi *et al*., 2019).

2.6 The ferric reducing antioxidant power (FRAP)

Oyaizu devised a method in 1986 that was used to evaluate the FRAP of the extracts. The FRAP was calculated using phosphate buffer, potassium ferricyanide, and extracts at various concentrations. Trichloroacetic acid was then added, and the mixture underwent centrifugation, following an incubation period. The absorbance was then determined at 700 nm after mixing ferric chloride with the top layer. The IC50 (mg/mL) value of the results was used to express the concentration at which 50% of the absorbance was achieved. The effectiveness of the extracts as antioxidants is indicated by this value (Oyaizu, 1986).

2.7 The ABTS antiradical scavenging assay

The ABTS assay, following the methodology introduced by Re *et al.* in 1999, involved the generation of ABTS+ cationic radicals by combining 2 mM ABTS with potassium persulfate for a duration of 24 hours. Subsequently, the resulting ABTS+ solution was diluted, and 150 µl of the extract or Trolox standard was added to 2850 µl of the solution. After an incubation period of 30 minutes, the absorbance was measured at 734 nm. The anti-radical power was expressed as the percentage of inhibition against ABTS+ radicals, and the IC50 values were determined as the concentrations at which 50% inhibition occurred (Re *et al*., 1999).

2.8 The total antioxidant capacity (TAC)

The TAC test was assessed using a method based on the reduction of Mo (VI) to Mo (V) and the formation of a green phosphate/Mo (V) complex at acidic pH. The extracts or standard antioxidants were mixed with a reagent solution, incubated at 95°C for 90 minutes, and then cooled. Absorbance was measured at 695 nm, and the results were expressed as mg ascorbic acid equivalents per gram of dry weight (mg AAE/g DW) (Prieto *et al*., 1999).

2.9 The statistical analysis

The data analysis involved the utilization of the One-way analysis of variance (ANOVA) test in the IBM SPSS statistics software (version 20.0, 2011). To evaluate the significance of variances between means, Duncan's multiple range test was implemented with a level of significance set at $p < 0.05$. Spearman's correlation coefficient (r) was employed to explore the associations between different

analytical parameters. Each experiment was replicated three times, and the results are reported as the mean ± standard deviation (SD).

3. Results and Discussion

3.1 HPLC-MS analysis

The analysis of the percentage areas of chemical compounds in different extracts, such as Thyme Ethyl Acetate Extract (EAET), Thyme Methanolic Extract (EMT), and others, reveals significant variations in sample composition. The table 1 and Figures (1 – 6) below provide a detailed overview of the identified molecules.

Table 1. percentage areas of chemical compounds in different Soxhlet extracts.

					% Area					
No	**RT**	**UV**	**m/z**	**Proposed compounds**	**EMO**	**EMT**	**EAQO**	**EAQT**	**EAEO**	**EAET**
1	2.79	278	-	*nd*	-	-	-	-	6.74	
2	4.58	272	125.1	*Pyrogallic Acid*	-	-	-	-	15.62	
3	7.54	280	256.12	*Pinocembrine*	-	2.12	-	3.76		
4	11.78	220, 320	367.1	*Acide4-O-Feruloylquinic*	-		2.79		-	8.89
5	12.84	272, 332	593.14	*Vitexin-4-O-glucoside*	4.41	5.4	12.5	10.41	-	
6	16.91	255, 348	163.1	*p-coumarique*	-		5.69		-	2.29
7	17.3	255, 355	447.12	*Quercitrin*	-	4.13	7.87	3.11	-	
8	18.9	285	609.17	*Hesperidin*	-		-		26.11	20.61
9	19.33	250, 310sh	771	*Quercetin-3-O-rutinoside-7-glucoside*	5.44	5.32	3.81	3.44		9.85
10	20.72	286, 345	579.12	*Naringine*	3.51	20.85	21.45	30.25	2.24	
11	21.4	255, 355	609.1	*Rutin*	-	2.14	2.99	11.14	-	
12	22.06	250, 355	623.12	*Isorhamnetin-3-O-rutinoside*	-	-	6.14		-	
13	25.19	255, 355	301.1	*Quercetin*	-	-	18.45	3.28	-	
14	25.88	290, 340	-	*nd*	8.55	-	1.02	-	-	
15	26.28	235, 342	-	*nd*	-	4.36	5.05	-	-	3.24
16	27.38	270, 335	269.12	*Apigenne*	15.32	9.7	1.21	-	-	
17	28.16	260	337.4	*Licoflavone C*	50.85	9.95	1.14	-	1.11	17.65
18	28.45	247, 282, 333	-	*nd*	-	19.85	-	-	-	10.11
19	29.15	262	337.14	*Licoflavone C isomer*	-	-	-	-	30.21	

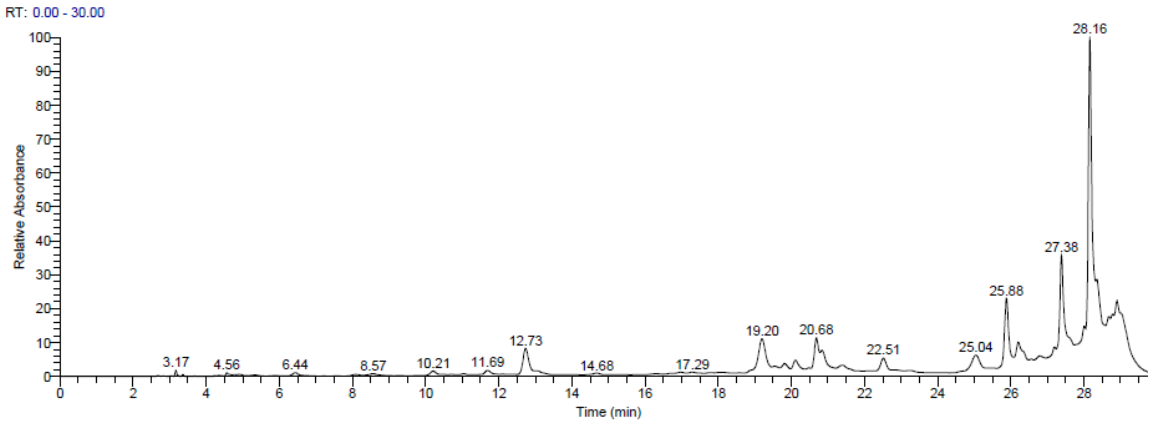


Figure 1. Chromatographic profile of methanolic extract of *O. grossii*.

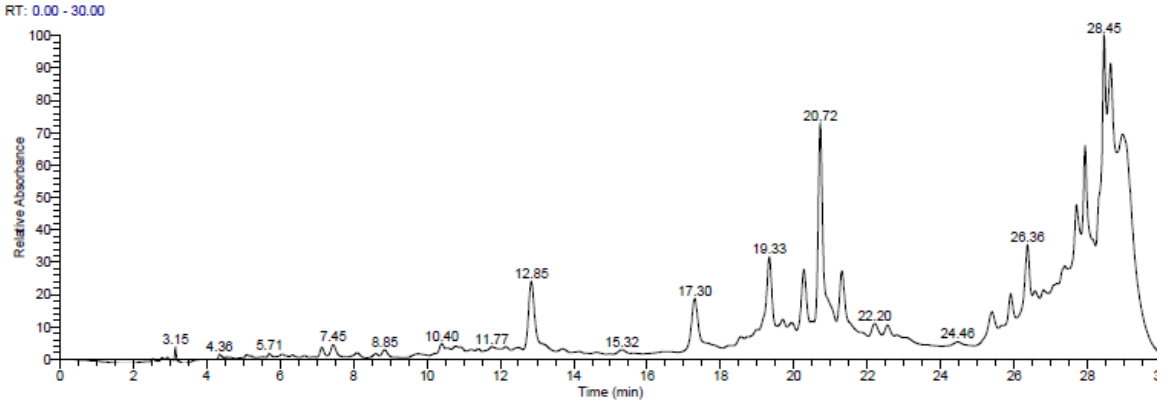


Figure 2. Chromatographic profile of methanolic extract of *T. pallidus*.

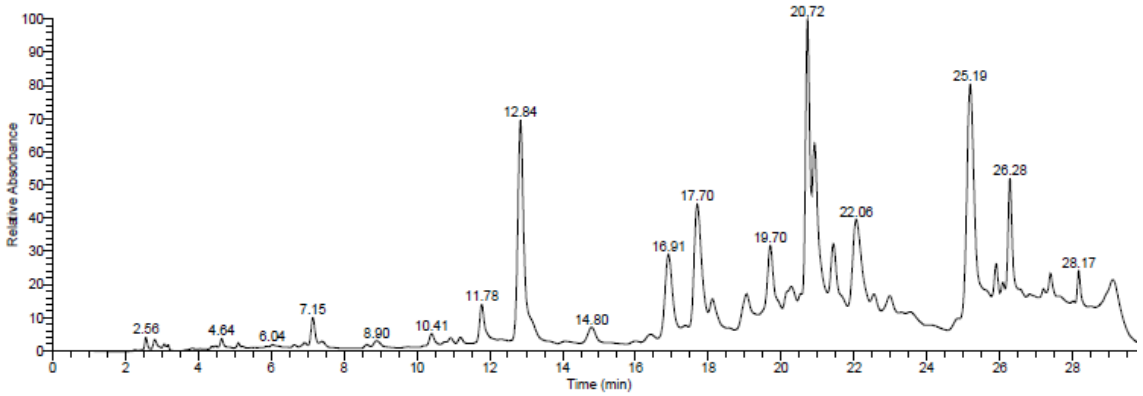


Figure 3. Chromatographic profile of aqueous extract of *O. grossii*

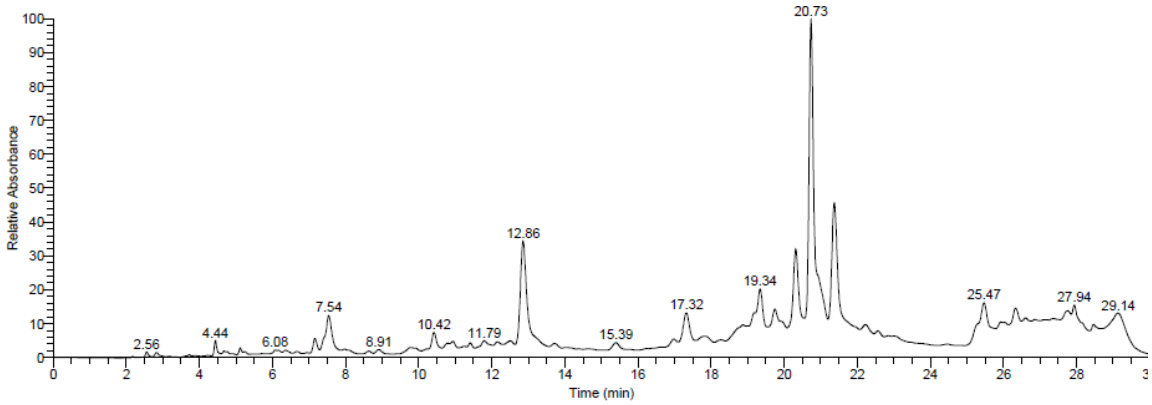


Figure 4. Chromatographic profile of aqueous extract of *T. pallidus*.

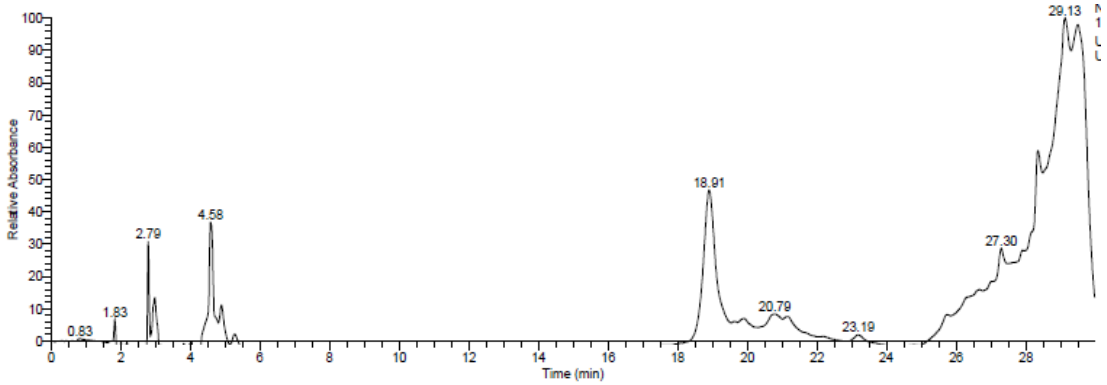


Figure 5. Chromatographic profile of ethyl acetate extract of *O. grossii*.

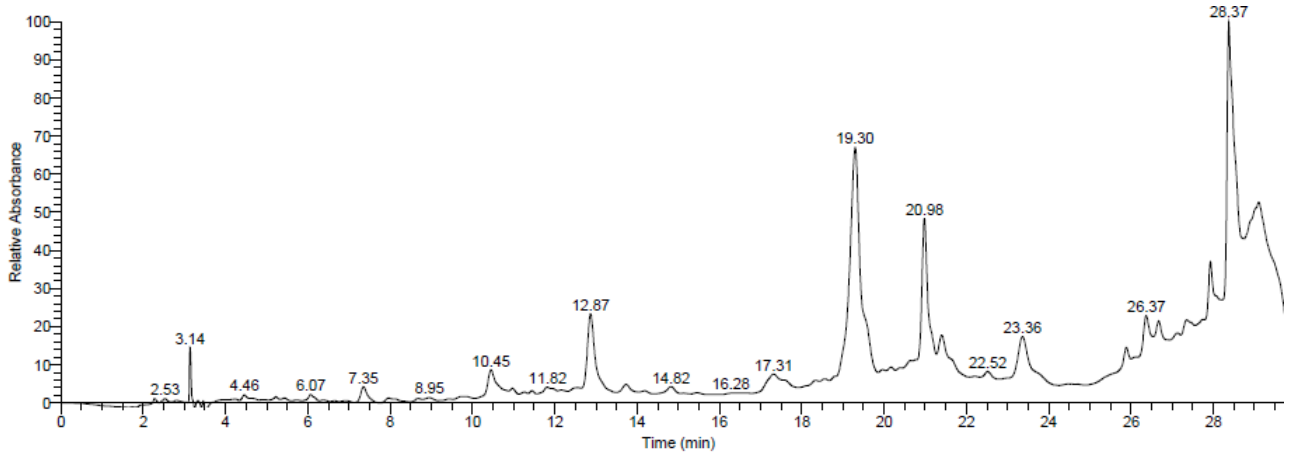
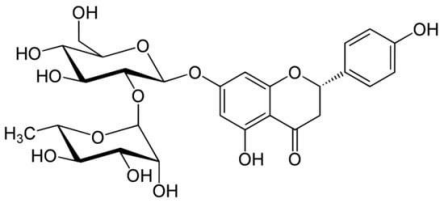
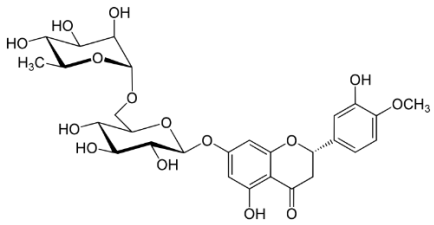


Figure 6. Chromatographic profile of ethyl acetate extract of *T. pallidus*.

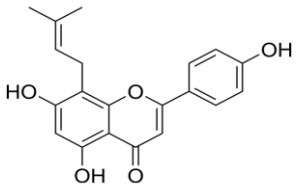
A thorough analysis of the percentage areas for each chemical compound in different extracts offers insightful observations. Pyrogallic acid stands out in the Thyme Ethyl Acetate Extract (EAET), constituting 15.62% of this extract, suggesting significant prevalence. Pinocembrin is notable in the Thyme Methanolic Extract (EMT) (2.12%) and Thyme Aqueous Extract (EAQT) (3.76%), indicating a specific distribution between thyme and Origanum extracts. Vitexin-4-O-glucoside, present in several extracts, exhibits variable concentrations, with a predominance in EAQT (10.41%) and EMT (5.4%). Some extracts are distinguished by specific compounds, such as hesperidin in Origanum Ethyl Acetate Extract (EAEO) (26.11%) and naringin in EAQT (30.25%) and EMT (20.85%). Interestingly, the isomer of licoflavone C predominates in EAET (30.21%). These diverse and significant variations in chemical composition among the extracts underscore the importance of these molecules, not only in contributing to the antioxidant activity but also in exploring potential avenues for various applications and outcomes(Sabbahi *et al.*, 2020). These diverse and significant variations in chemical composition among the extracts underscore the importance of these molecules, not only in contributing to the antioxidant activity but also in exploring potential avenues for various applications and outcome(Ognik *et al.*, 2016).



Molecular Structure of Naringin



Molecular Structure of Hesperidin



Molecular Structure of licoflavone C

Scheme 1. Molecular Structure of major compounds in *O. grossii* and *T. pallidus*.

3.2 The total flavonoids content (TRC) and the total phenolic content (TPC)

In this study, the yield of the distilled water extract of thyme leaves was higher compared to that of oregano leaves. Both plants exhibited a similar decreasing trend in yields. For oregano extracts, the order of yields in percentage (%) was as follows: 2.5% aqueous > 1.3% methanol > 1% ethyl acetate. Similarly, for thyme extracts, the yields were 4.5% for the aqueous extract > 1.3% for the methanolic extract > 0.9% for the ethyl acetate extract. This indicates a decrease in yield as the polarity of the solvent decreases (**Table 2**).

Table 2. The extraction rate, total phenolic, and flavonoid content of oregano and thyme in aqueous, methanolic, and ethyl acetate fractions

Sample	Yield%		Total Flavonoid Content (mg RE/g DW)		Total Phenolic Content (mg GAE/g DW)	
	Oregano	Thyme	Oregano	Thyme	Oregano	Thyme
AQ	2.5%	4.5%	463.56±0.02^{b}	356.56±3.28^{b}	171.45±1.21^{c}	163.069±1.02^{c}
ME	1.3%	1.3%	372.97±1.96^{a}	114.51±1.73^{a}	292.91±1.51^{a}	284.3±1.78^{a}
AE	1%	0.9%	172.15±2.55^{c}	88.541±1.24^{c}	3.804±0.22^{b}	3.565±0.28^{b}

GAE: Gallic Acid Equivalent, DW: Dry Weight, RE - Rutin Equivalent, Data are reported as mean values ± SD of three measurements. Means were significantly different when P<0.05; values followed by different letters are significantly different.

The total phenolic and flavonoid contents of *Origanum grossii* and *Thymus pallidus* extracts were determined. The results (Table 2) indicate that methanol extracts showed significantly higher phenolic content for both plants (*Origanum grossii*: 292.91±1.51 mg GAE/g DW, *Thymus pallidus*: 284.3±1.78 mg GAE/g DW). Water extracts exhibited moderate phenolic content (*Origanum grossii*: 171.45±1.21 mg GAE/g DW, *Thymus pallidus*: 163.069±1.02 mg GAE/g DW), while ethyl acetate extracts had the lowest phenolic content (*Origanum grossii*: 3.804±0.22 mg GAE/g DW, *Thymus pallidus*: 3.565±0.28 mg GAE/g DW).

Phenolic compounds are widely recognized as the primary contributors to the antioxidant capacity of plants (E. Ouadi *et al*., 2021). Our study's results regarding the phenolic content of *Thymus* spp. and *Origanum* spp. extracts align with previous literature findings, the higher phenolic content was observed in the methanol extract, when compared to water and, ethyl acetate extracts is consistent with studies conducted on Thymus species. For instance, (Zanotto *et al.*, 2023) reported higher phenolic content in methanol extracts of *Thymus vulgaris*, supporting our findings. Additionally, (Stagos *et al.*, 2012) analyzed the phenolic content of Thymus vulgaris extracts and found comparable results to our water extract.

Regarding the total flavonoid contents, water extracts displayed the highest amounts (*Origanum grossii*: 463.56±0.02 mg RE/g DW, *Thymus pallidus*: 356.56±3.28 mg RE/g DW). On the other hand, the minimum amounts were observed in the ethyl acetate extracts (*Origanum grossii*: 172.15±2.55 mg RE/g DW, *Thymus pallidus*: 88.541±1.24 mg RE/g DW). Our results indicate significant variations among the extracts. This finding is in line with studies conducted on *Thymus* species. For example, (Taghouti *et al.*, 2020) investigated the flavonoid content of *Thymus citriodorus* extracts and reported similar variations among different solvent extracts, with higher flavonoid content in water extract.

The role of polyphenols, including phenolic compounds and flavonoids, in the antioxidant activities of plants has been well-documented in the literature (Bogoyavlenskiy *et al.*, 2015; Hadda *et al.*, 2013). The redox properties of polyphenols enable them to act as effective reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators, contributing to their antioxidant potential (Pejatovic *et al.*, 2017). The literature provides supporting evidence for our findings. For example, (Zanotto *et al.*, 2023) evaluated the phenolic content of *Origanum vulgare* extracts and reported comparable results to our methanol extract. Additionally, previous studies on *Origanum vulgare* conducted by (Amarowicz *et al.*, 2009) and (Cioroi & Dumitriu, 2009) demonstrated variations in phenolic and flavonoid contents among different solvent extracts, with higher contents observed in methanol extracts and water extracts, respectively. Overall, our findings regarding the phenolic and flavonoid contents of *Thymus*

spp. and *Origanum* spp. extracts align with existing literature, confirming the significant contribution of these compounds to the antioxidant activities of the extracts. The variations observed among different solvent extracts further highlight the importance of extraction methods in optimizing the extraction of bioactive compounds with antioxidant potential (Nazir *et al*., 2017).

3.3 FRAP, DPPH, and ABTS assays.

The Table 3 presents the antiradical abilities of aqueous, methanolic, and ethyl acetate fractions of Oregano and Thyme, compared to standard antioxidants, using FRAP, DPPH, and ABTS assays.
For the ferric reducing power ability, the results ranged from 0.101 ± 0.007 to 0.492 ± 0.01 mg/mL for *Origanum grossii*, and from 0.188 ± 0.006 to 0.420 ± 0.022 mg/mL for *Thymus pallidus*. In the case of oregano, the methanolic extracts exhibited similar ferric reducing power capacity compared to the water extracts, and their results were significantly higher than that of the ethyl acetate extract (0.492 ± 0.01 mg/mL), although still lower than the standard antioxidant used. For thyme extracts, the methanol extract showed the highest value (0.188 ± 0.006 mg/mL), followed by the water extract (0.204 ± 0.009 mg/mL), and the ethyl acetate extract (0.420 ± 0.022 mg/mL). The results of the ferric reducing power assay align with previous studies. (Hyun et al., 2014) evaluated the ferric reducing activity of *Thymus quinquecostatus* extracts and observed similar reducing power to our methanol and water extracts of *Thymus* spp. Additionally, in a study by (Kouri *et al*., 2007), the ferric reducing power of *Origanum dictamnus* extracts was investigated, and the findings are consistent with our results, showing higher reducing power in methanolic and aqueous extracts compared to ethyl acetate extract.

Table 3. The inhibitory abilities of the water, methanolic, and ethyl acetate fractions of Oregano and Thyme were assessed in comparison to standard molecules using various antioxidant assays

Sample	FRAP		DPPH		ABTS	
	Oregano	Thyme	Oregano	Thyme	Oregano	Thyme
Water	0.134±0.01^{a}	0.204±0.009^{a}	0.084±0.007^{a}	0.216±0.018^{c}	0.139±0.007^{a}	0.112±0.01^{b}
ME	0.101±0.007^{ac}	0.188±0.006^{a}	0.067±0.003^{a}	0.153±0.012^{a}	0.161±0.007^{a}	0.089±0.01^{a}
AE	0.492±0.01^{b}	0.420±0.022^{b}	0.359±0.026^{b}	0.227±0.033^{b}	0.863±0.003^{b}	0.131±0.03^{b}
BHT	-		0.119±0.002^{a}		-	
Quercetin	0.030±0.006^{c}		-		-	
Trolox	-		-		0.126±0.005^{a}	

GAE: Gallic Acid Equivalent, DW: Dry Weight, RE - Rutin Equivalent, Data are reported as mean values ± SD of three measurements. Means were significantly different when P<0.05; values followed by different letters are significantly different.

Table 3 displays also, the DPPH radical scavenging activities of the extracts from oregano. All extracts exhibited antioxidant activity in this assay, with IC50 values ranging from 0.067 to 0.359 mg/mL for the methanol and ethyl acetate extracts, respectively. The IC50 values of the methanolic extract (0.067 ± 0.003 mg/mL) and water extract (0.084 ± 0.007 mg/mL) were significantly superior to the positive control BHT (0.119 ± 0.002 mg/mL). For the thyme extract, the IC50 values ranged from 0.153 to 0.227 mg/mL for the methanol and ethyl acetate extracts, respectively. The IC50 value of the methanolic extract (0.153 ± 0.012 mg/mL) was significantly similar to that of the positive control BHT. The obtained results regarding the antioxidant activities of *Thymus* spp. and *Origanum* spp. extracts are consistent with previous studies in the literature. Various research articles have highlighted the potent antioxidant properties of these plants and their bioactive compounds. In support of our findings, (Nikolić *et al*., 2014) evaluated the antioxidant activities of different *Thymus* species using the DPPH

assay. The authors reported significant free radical scavenging activity in the methanolic extracts, which is in line with our results. Furthermore, Nakiboglu *et al.* investigated the antioxidant potential of *Origanum vulgare*, and *Origanum sipyleum* extracts using the DPPH assay, and the obtained IC50 values were comparable or less than the ones we obtained for the methanolic and aqueous extracts of *Origanum grossii* (Nakiboglu *et al*., 2007).

In the ABTS assay, all extracts obtained from oregano, as shown in Table 2, exhibited antioxidant activity. The IC50 values for water and ethyl acetate extracts ranged from 0.139 to 0.863 mg/mL, respectively. The IC50 value of the water extract (0.139 ± 0.007 mg/mL) and the methanol extract (0.161 ± 0.007 mg/mL) were similar to the positive control, Trolox (0.126 ± 0.005 mg/mL), indicating comparable antioxidant potency. Regarding thyme extract, the results ranged from 0.089 to 0.131 mg/mL for water and ethyl acetate extracts, respectively. The IC50 value of the aqueous extract (0.089 ± 0.01 mg/mL) showed significantly higher antioxidant activity than the positive control.

3.4 The total antioxidant capacity

The total antioxidant capacity of oregano and thyme extracts was determined by measuring the formation of phosphomolybdenum complexes. Figure 7 presents the results illustrating the total antioxidant capacity of these extracts, expressed in terms of ascorbic acid equivalents (mg AAE/g DW). For oregano, the methanol extract exhibited the highest total antioxidant capacity, measuring 945.43±7.98 mg AAE/g DW. It was followed by the aqueous extract with a capacity of 550.45±2.97 mg AAE/g DW, and then the ethyl acetate extract with a capacity of 37.07±0.25 mg AAE/g DW. Similar results were observed for thyme extract, with the methanol extract displaying a total antioxidant capacity of 928.407±4.41 mg AAE/g DW, followed by the aqueous extract with a capacity of 488.30±0.17 mg AAE/g DW, and finally the ethyl acetate extract with a capacity of 27.73±0.21 mg AAE/g DW.

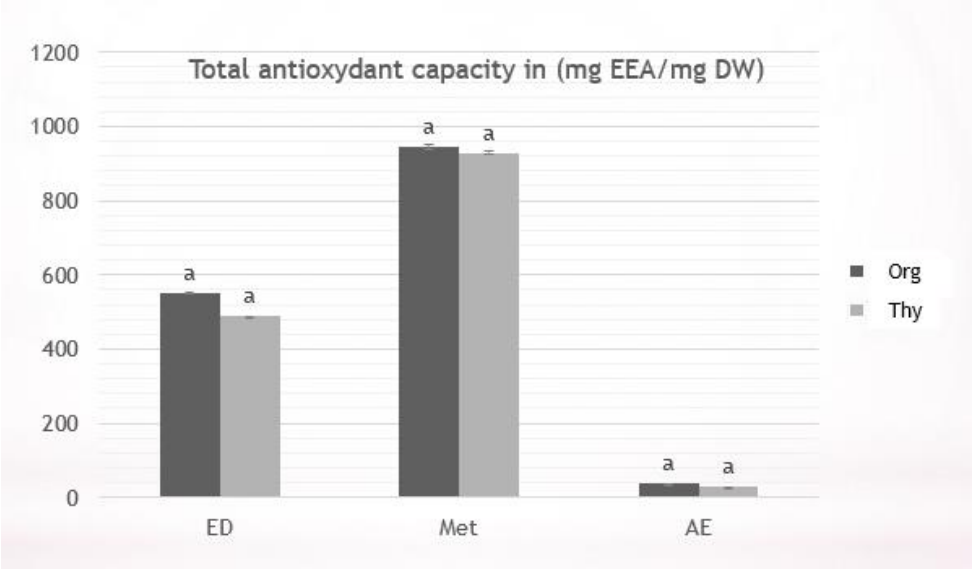


Figure 7. Total Antioxidant activity of aqueous, methanolic and ethyl acetate fractions of Oregano and Thyme
GAE: Gallic Acid Equivalent, DW: Dry Weight, RE - Rutin Equivalent, Data are reported as mean values ± SD of three measurements. Means were significantly different when P<0.05; values followed by different letters are significantly different

The determination of total antioxidant capacity using the phosphomolybdenum method has also been widely employed in the assessment of plant extracts. Our results indicating the highest total antioxidant

capacity in the methanolic extracts of *Thymus pallidus*. and *Origanum grossii*. are supported by studies conducted by (Amarowicz *et al*., 2009) and (Nakiboglu *et al*., 2007) on *Thymus vulgaris* and *Origanum vulgare*, respectively. Moreover, the observed variations in antioxidant activities among different solvent extracts are consistent with the findings of several studies. (Hyun *et al*., 2014) investigated *Thymus quinquecostatus* and found that different solvent extracts exhibited varying degrees of antioxidant activity, with methanol extracts showing the highest activity, followed by water and ethyl acetate extracts. Similarly, (Nakiboglu *et al*., 2007) evaluated the antioxidant activities of methanolic solvent extract of *Origanum vulgare* and reported significant differences, with methanol extracts showing the highest antioxidant potential.

3.5.The Spearman's correlation:

In **Table 5** the Spearman's correlation coefficients reveal the relationships between different antioxidant assays (AAT, FRAP, DPPH, ABTS) and two compound types (polyphenols and flavonoids). For polyphenols, a strong positive correlation (0.946**) indicates that higher polyphenol concentrations result in significantly increased antioxidant activity measured by AAT. Conversely, a strong negative correlation (-0.863**) is observed between polyphenols and FRAP, suggesting that higher polyphenol levels are associated with reduced ferric ion reducing power. The correlation between polyphenols and DPPH is weak (-0.074), while a moderate negative correlation (-0.601**) exists between polyphenols and ABTS, indicating a negative relationship between polyphenol content and ABTS radical scavenging activity.

Table 5. The Spearman's correlation correlations between the amount of total phenolic and flavonoid compounds and antioxidants employed.

	Coefficient de corrélation (Spearman)			
	AAT	FRAP	DPPH	ABTS
Polyphenols	0,946**	-0,863**	-0,074	-0,601**
Flavonoides	0,513**	-0,663**	-0,463*	-0,298

* significative La correlation at 0,05 (bilateral). ** La corrélation est significative au niveau 0,01 (bilatéral).

Regarding flavonoids, a moderate positive correlation (0.513**) is observed between flavonoids and AAT, indicating that higher flavonoid levels are associated with increased antioxidant activity measured by AAT. Similarly, a strong negative correlation (-0.663**) exists between flavonoids and FRAP, suggesting that higher flavonoid concentrations correspond to reduced ferric ion reducing power. Flavonoids also show a moderate negative correlation (-0.463*) with DPPH, implying that higher flavonoid levels may be linked to lower DPPH radical scavenging capacity. However, a weak negative correlation (-0.298) is observed between flavonoids and ABTS, indicating no significant relationship between flavonoid content and ABTS radical scavenging activity. The literature findings corroborate our study's results and provide additional support for the antioxidant activities observed in *Thymus* spp. and *Origanum* spp. extracts. The presence of bioactive compounds, such as phenolic compounds and flavonoids, in these plants is well-documented and known to contribute to their antioxidant properties. Therefore, our study reinforces the potential of *Thymus pallidus*. and *Origanum grossii*. as natural sources of antioxidants with possible health benefits.

Conclusion

In conclusion, this study aimed to determine the polyphenol and flavonoid content of *Thymus pallidus* and *Origanum grossii* extracts and comprehensively evaluate their antioxidant activity. The results of our study provide further evidence supporting the observed antioxidant properties of thyme and oregano extracts. The presence of bioactive compounds, particularly phenolic compounds and flavonoids, in these plants is well-established and known to contribute to their antioxidant effects. Thus, our findings reinforce the potential of *Thymus pallidus* and *Origanum grossii* as natural sources of antioxidants, which could have potential health benefits.

Looking ahead, future research could delve deeper into the specific mechanisms underlying the antioxidant activities of *Thymus pallidus* and *Origanum grossii* extracts. Additionally, exploring the potential applications of these extracts in various industries, such as food, pharmaceuticals, or cosmetics, could be an interesting avenue to pursue. Further studies might also consider investigating the synergistic effects of these extracts with other natural antioxidants or evaluating their efficacy in different models or conditions. Overall, the outcomes of this study provide a foundation for future research and emphasize the promising prospects of *Thymus pallidus* and *Origanum grossii* as valuable sources of antioxidants.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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