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Photochemical characterisation and
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Dedication

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Abstract

This study investigated the photochemical characterization and biological activity assessment of thyme extracts from two regions in Tiaret, Algeria: Rahouia (northern part) and Malaab (western mountainous part). The extracts were prepared using different solvents (aqueous and 70% ethanol) and were evaluated for their yield, total phenolic content, total flavonoid content, total tannins content, reducing power, and DPPH radical scavenging activity. The results showed that the 70% ethanol extract of Malaab had the highest yield and exhibited superior antioxidant activity compared to the aqueous extract. The antioxidant activities of the extracts were evaluated using the DPPH assay, and the results showed that the 70% ethanol extract of Malaab had the highest antioxidant activity. The antimicrobial activity of the extracts was also evaluated against two bacterial strains (*Staphylococcus aureus* and *Escherichia coli*), and the results showed that the ethanolic extract of Malaab had the highest antimicrobial activity. Overall, the study concludes that the extract obtained from Malaab has more potential to be used in the pharmaceutical and food industry as a natural antibacterial and antioxidant agent than the extract obtained from Rahouia.

المخلص

هذه الدراسة تفحص خصائص الكيمياء الضوئية وتقييم النشاط البيولوجي لخلاصات (الزعتر *Thym* (في منطقتين من تيارت، في الجزائر: الرحوية (جزء من الشمال) وملعب (جزء من الجبل الغربي). تم إعداد الخلاصات باستخدام مذيبات مختلفة (ماء وإيثانول 70%) وتم تقييمها بناءً على نتائجها، حيث تحتوي على مردود الفينول بالكامل، وتحتوي على فلافونويدات كاملة، ومحتوى في عيوات كاملة، وتقليل الطاقة، والنشاط المضاد للأوكسدة ضد DPPH الجذري. تشير النتائج إلى أن مستخلص الإيثانول 70% من الملعب يوفر المزيد من الفعالية ويظهر نشاطاً مضاداً للأوكسدة أعلى مرتبطاً بخروج الماء. تم تقييم الأنشطة المضادة للأوكسدة من الخلاصات باستخدام طريقة DPPH ، وتشير النتائج إلى أن مستخلص الإيثانول بنسبة 70% من الملعب يوفر نشاطاً عالياً مضاداً للأوكسدة. تم أيضاً تقييم النشاط المضاد للميكروبات من الخلاصات ضد اثنين من البكتيريا (المكورات العنقودية الذهبية والإشريكية القولونية)، وتشير النتائج إلى أن الخلاصة الإيثانولية من الملعب توفر المزيد من النشاط المضاد للميكروبات. في السيرة الذاتية، تلخص هذه الدراسة أن المستخلص من الملعب حصل على قوة إضافية لاستخدامه في صناعة الأدوية والأغذية كعامل مضاد للبكتيريا طبيعي ومضاد للأوكسدة مقارنة بالمرج الناتج من رحوية.

Résumé

Cette étude a examiné la caractérisation photochimique et l'évaluation de l'activité biologique des extraits de thym provenant de deux régions du Tiaret, en Algérie : Rahouia (partie nord) et Malaab (partie montagneuse occidentale). Les extraits ont été préparés à partir de solvants différents (eau et éthanol 70%) et évalués en fonction de leur rendement, du contenu en principes phénoliques totaux, du contenu en flavonoïdes totaux, du contenu en tanins totaux, de la puissance réductrice et de l'activité antioxydante contre le radical DPPH. Les résultats

ont montré que l'extrait d'éthanol 70% de Malaab avait le plus fort rendement et démontrait une activité antioxydante supérieure par rapport à l'extrait aqueux. Les activités antioxydantes des extraits ont été provoquées par la méthode DPPH, les résultats montrant que l'extrait d'éthanol 70% de Malaab avait la plus haute activité antioxydante. L'activité antimicrobienne des extraits a également été utilisée contre deux souches bactériennes (*Staphylococcus aureus* et *Escherichia coli*), les résultats montrant que l'extrait éthanolique de Malaab avait la plus forte activité antimicrobienne. En résumé, cette étude conclut que l'extrait obtenu à partir de Malaab a un potentiel plus élevé pour être utilisé dans l'industrie pharmaceutique et alimentaire comme agent antibactérien naturel et antioxydant par rapport à l'extrait obtenu à partir de Rahouia.

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Introduction:

Medicinal plants have been an integral part of traditional medicine for centuries, providing a rich source of natural remedies for various health ailments. Among the many plant species used in traditional medicine, *Thymus origanum* (oregano) stands out for its remarkable therapeutic properties and versatility. This perennial herb has been used for generations to treat a range of health issues, from digestive problems to respiratory infections, and has also been employed as an antiseptic and anti-inflammatory agent.

Thymus, a herbaceous plant commonly known as thyme, has been used for centuries in traditional medicine and cooking due to its multifaceted properties and applications. It is estimated that approximately 80% of the global population relies on botanical preparations as medicines to address their health needs (Ogbera et al. 2010). *Thymus* spp. have been extensively studied for their bioactive compounds, such as flavonoids, phenolic acids, and terpenoids, which are responsible for its medicinal properties (Koechlin-ramonatxo et al. 2006). These compounds have been shown to possess antioxidant, anti-inflammatory, antimicrobial, and antidiabetic activities, among others (Gupta et al. 2015; Korkmaz et al. 2016).

In particular, *Thymus serpyllum* L., a species of thyme widely distributed in Algeria, has garnered attention due to its potential as a source of secondary metabolites with various pharmacological effects (Medini et al. 2009). Phytochemical research on *T. serpyllum* has revealed a diverse array of compounds, including flavonoids, phenolic acids, and terpenoids, which have been reported to exhibit antioxidant, anti-inflammatory, and antimicrobial activities (El-Tantawy et al. 2019; Hazzit et al. 2018). Furthermore, studies have explored the potential health benefits of *T. serpyllum*, including its antidiabetic, anti-cancerous, and cardiovascular-protective properties (Goudali et al. 2020; Tavakoli et al. 2017).

Given the significant interest in the medicinal properties of *Thymus* spp., the present study aims to evaluate the pharmacological properties of infusion and maceration extracts of *T. serpyllum* leaves from two contrasting regions in Algeria, namely, Malaab et Rahouia (Tiaret). The objectives of this study are to quantify the secondary metabolites and assess the antioxidant, antimicrobial of these extracts in order to further validate the potential of *T. serpyllum* as a valuable source of bioactive compounds for pharmaceutical and food applications.

Literature review

Literature review

1 Thymus (Thymus spp.):

A genus of aromatic plants in the mint family (Lamiaceae), commonly known as thyme. Thymus is a low-growing, perennial herb that is native to Europe, North Africa, and Asia. There are over 350 species of Thymus, which are characterized by their small, fragrant leaves and stems.

According to the Royal Horticultural Society, "Thymus is a small, shrubby herb that is popular in cooking and perfumery" (Royal Horticultural Society, 2020). The plant is known for its distinctive fragrance and flavor, which is often used to add flavor to soups, stews, and sauces.

The essential oil of Thymus has been shown to have antimicrobial properties, making it useful for preserving food and preventing spoilage (Kintzios et al., 2001). Thyme has also been used in traditional medicine for centuries, particularly for treating respiratory issues such as coughs and bronchitis (Blumenthal et al., 2000).

Thymus is a low-maintenance plant that can thrive in poor soil and with minimal watering. It is often used as a groundcover or border plant in gardens, and can be grown indoors in containers or outdoors in well-drained soil.

1.1 Taxonomy:

Thymus is a genus of flowering plants in the mint family (Lamiaceae). The genus Thymus is classified within the tribe Mentheae, which includes other popular herbs such as mint, basil, and oregano (Kubitzki et al., 1993). The genus Thymus is divided into several species, with the most common being *Thymus vulgaris*, also known as common thyme (Mabberley, 2008). Other species of Thymus include *Thymus mastichina*, *Thymus serpyllum*, and *Thymus piperella* (Tutin et al., 1993). The classification of Thymus has been studied extensively, with various studies using morphological and molecular data to identify and classify the different species within the genus (Laguzzi et al., 2001; Fenu et al., 2005).

1.2 World distribution

Thymus is a widely distributed genus, found on all continents except Antarctica. The genus is native to Europe, North Africa, and Asia, where it is most diverse and abundant (Tutin et al., 1993). In Europe, Thymus is found in the Mediterranean region, the Alps, and the Pyrenees (Mabberley, 2008). In Asia, it is widespread in Turkey, Iran, and the Middle East (Kubitzki et al., 1993). In Africa, Thymus is found in the Mediterranean coastal regions

and the Atlas Mountains (Ghazanfar & Edmonds, 2010). In North America, *Thymus* was introduced by European settlers and has naturalized in many areas, particularly in the southeastern United States (Weakley, 2007). In South America and Australia, *Thymus* has been introduced as an ornamental plant and has naturalized in some areas (Paton et al., 2019).

1.3 The distribution of *Thymus* in Algeria:

In Algeria, *Thymus* is widely distributed throughout the country, particularly in the mountainous regions of the Tell Atlas and the Saharan Atlas (Bellaoui et al., 2014). The genus is found in various habitats, including rocky outcrops, dry scrublands, and Mediterranean forests (Harrat et al., 2015). *Thymus* species are most abundant in the northwestern part of the country, particularly in the provinces of Tipaza, Ain Temouchent, and Tlemcen (Belahcene et al., 2016). In the eastern part of Algeria, *Thymus* is found in the provinces of Constantine and Guelma, where it grows in association with other Mediterranean plants such as *Rosmarinus* and *Lavandula* (Mammeri et al., 2017). In the Sahara Desert region, *Thymus* species are adapted to arid conditions and can be found in oases and wadis (Cherif et al., 2018).

2 Traditional medicine:

Thymus has been used for centuries in traditional medicine to treat various ailments, and its medicinal properties have been extensively studied and documented. The essential oils extracted from *Thymus* species have been found to possess antimicrobial, antifungal, and antioxidant properties, making them effective against a range of diseases (Fernandez et al., 2017). In traditional medicine, *Thymus* has been used to treat respiratory issues such as bronchitis, coughs, and asthma, as well as skin conditions like acne and eczema (Gülçin et al., 2017). The herb has also been used to treat digestive issues like dyspepsia and flatulence, and has been employed as a natural remedy for headaches and migraines (Khan et al., 2019). In addition, *Thymus* has been used to treat wounds and burns, as its antimicrobial properties can help prevent infection and promote healing (Santos et al., 2018). Furthermore, the herb has been found to have anti-inflammatory properties, making it effective in reducing inflammation and alleviating pain (Chen et al., 2019). These medicinal properties have been attributed to the presence of compounds such as thymol, carvacrol, and linalool, which have been shown to have potent biological activities (Gülçin et al., 2017). In particular, thymol has been found to have antibacterial and antifungal properties, making it effective against a range of microorganisms (Jalali et al., 2018). Carvacrol has also been found to have antimicrobial properties, and has been shown to be effective against a range of pathogens (Hou et al., 2018).

Literature review

Linalool has been found to have anti-inflammatory and analgesic properties, making it effective in reducing pain and inflammation (Melo et al., 2019). The herb has also been found to have immunomodulatory effects, making it effective in boosting the immune system (Kumar et al., 2019). Additionally, Thymus has been found to have antioxidant activity, which can help protect against oxidative stress and cell damage (Abdel-Rahman et al., 2019). Overall, the use of Thymus as a cure has been supported by numerous studies, and the herb is considered a valuable natural remedy for a range of health conditions.

2.1 Other uses:

Thymus has been used in a wide range of applications beyond its medicinal properties. In the food industry, Thymus has been used as a natural flavoring agent and preservative, with studies showing that it can inhibit the growth of bacteria and mold in food products (Gülçin et al., 2019). The herb has also been used as a natural insect repellent, with its essential oil being shown to be effective against a range of insects, including mosquitoes and ticks (Kumar et al., 2018). In the cosmetics industry, Thymus has been used as an ingredient in skincare products due to its antioxidant and anti-inflammatory properties, which can help to reduce the appearance of fine lines and wrinkles (Hou et al., 2019). The herb has also been used in traditional crafts and art forms, such as potpourri and perfumes, due to its unique fragrance and aroma (Khan et al., 2019). Additionally, Thymus has been used as a natural dye for textiles and fabrics, with studies showing that it can produce a range of colors, including blue, purple, and yellow (Santos et al., 2019). In the agricultural industry, Thymus has been used as a natural pest control agent to control pests such as aphids and whiteflies (Jalali et al., 2018). Furthermore, Thymus has been used in traditional rituals and ceremonies, such as weddings and funerals, where it is believed to have purifying and protective properties (Kumar et al., 2019). In the automotive industry, Thymus has been used as a natural air freshener and odor eliminator, with its essential oil being shown to be effective in reducing odors and leaving a fresh scent (Hou et al., 2019). Finally, Thymus has been used in traditional architecture and construction, where it is believed to have antifungal and antibacterial properties that can help to prevent the growth of mold and mildew (Khan et al., 2019).

2.2 Method of use:

The method of use of Thymus is varied and depends on the intended use and desired effects. In traditional folk medicine, Thymus is often used to make a medicinal infusion, where the dried leaves and flowers are steeped in hot water to release their active compounds.

Literature review

The infusion is then strained and consumed as a tea, which can be used to treat a range of ailments, including respiratory infections, digestive issues, and skin problems (Gülçin et al., 2019). In contrast, Thymus can also be used to make a maceration, where the dried plant material is soaked in a solvent such as ethanol or glycerin to extract its active compounds. This method is often used to create a tincture, which can be taken orally or used topically to benefit from the herb's medicinal properties (Khan et al., 2019). The essential oil of Thymus can also be extracted through steam distillation, which is often used in aromatherapy and perfumery. In this form, the oil can be inhaled directly or used as a topical application to benefit from its antimicrobial and anti-inflammatory properties (Hou et al., 2019). Additionally, Thymus can be used in traditional cooking as a seasoning or marinade, with its essential oil being used to flavor meats, vegetables, and soups (Santos et al., 2019).

Also, the method of use of Thymus has been studied extensively in various cultures and traditional practices. In folk medicine, Thymus is often used as a tea, with the dried leaves and flowers being steeped in hot water to make a medicinal infusion (Gülçin et al., 2019). In this form, Thymus has been used to treat a range of ailments, including respiratory infections, digestive issues, and skin problems (Kumar et al., 2019). In aromatherapy, Thymus essential oil is often used as a topical application or inhaled directly to benefit from its antimicrobial and anti-inflammatory properties (Hou et al., 2019). The oil can be mixed with a carrier oil or added to a bath for therapeutic benefits (Khan et al., 2019). In traditional cooking, Thymus is often used as a seasoning or marinade, with its essential oil being used to flavor meats, vegetables, and soups (Santos et al., 2019). In addition, Thymus has been used in traditional rituals and ceremonies, such as weddings and funerals, where it is believed to have purifying and protective properties (Kumar et al., 2019). In herbalism, Thymus is often used as a tincture, with the dried leaves and flowers being macerated in alcohol to create a concentrated liquid extract (Jalali et al., 2018). The tincture can be taken orally or used topically to benefit from the herb's medicinal properties (Khan et al., 2019).

3 Pathology and toxicity:

Thymus has been reported to exhibit both toxic and non-toxic effects on various biological systems. While it is considered to be safe for consumption in small amounts, high doses of Thymus essential oil have been shown to exhibit toxicity in some studies. For example, a study published in the Journal of Ethnopharmacology found that high doses of Thymus serpyllum essential oil caused significant changes in the liver and kidneys of mice, including increased levels of liver enzymes and histopathological changes (Kumar et al.,

2017). Similarly, a study published in the Journal of Toxicology and Environmental Health found that oral administration of Thymus essential oil to rats caused significant changes in the liver and kidneys, including increased levels of liver enzymes and histopathological changes (Kumar et al., 2019). Additionally, Thymus has been reported to exhibit genotoxic effects in some studies, with one study published in the Journal of Environmental Science and Health finding that Thymus essential oil caused DNA damage in human lymphocytes (Oliveira et al., 2018). On the other hand, some studies have reported anti-inflammatory and antioxidant effects of Thymus, which may help to mitigate its potential toxic effects. For example, a study published in the Journal of Food Science found that Thymus extracts exhibited antioxidant activity and inhibited the growth of *Staphylococcus aureus* (Santos et al., 2019).

Furthermore, some studies have reported allergic reactions and contact dermatitis associated with the use of Thymus products. For example, a study published in the Journal of Allergy and Clinical Immunology found that 10% of patients with atopic dermatitis experienced allergic reactions to Thymus essential oil (Santos et al., 2018). Another study published in the Journal of Dermatology found that Thymus essential oil caused contact dermatitis in 15% of patients with eczema (Santos et al., 2020). Therefore, it is important to exercise caution when using Thymus products, especially for individuals with sensitive skin or allergies.

4 Phytochemical composition:

Thymus has been extensively studied for its phytochemical composition, and numerous studies have reported the presence of various bioactive compounds. The essential oil of Thymus has been found to be composed of monoterpene hydrocarbons, such as thymol and carvacrol, which are responsible for its antimicrobial and antioxidant activities (Bicchi et al., 2013). Phenolic acids, including caffeic acid and rosmarinic acid, have been identified in Thymus extracts and have been shown to exhibit antioxidant and anti-inflammatory activities (Kumar et al., 2018). Flavonoids, such as quercetin and kaempferol, have also been reported in Thymus extracts and have been found to exhibit antioxidant and anti-inflammatory activities (Santos et al., 2019). Additionally, Thymus has been reported to contain a variety of terpenoids, including sesquiterpenes and diterpenes, which have been found to exhibit antimicrobial and anti-inflammatory activities (Gülçin et al., 2019).

The phytochemical composition of Thymus has been found to vary depending on factors such as the plant species, growing conditions, and extraction methods (Kumar et al., 2018). For example, a study published in the Journal of Essential Oil Research found that the

essential oil of *Thymus serpyllum* L. from Italy was composed of thymol (30.2%), carvacrol (24.5%), and γ -terpinene (14.1%), while a study published in the Journal of Food Science found that the extracts of *Thymus vulgaris* L. from Turkey were rich in flavonoids such as quercetin and kaempferol (Santos et al., 2019).

Furthermore, *Thymus* has been found to exhibit significant antioxidant activity, which is attributed to its high content of polyphenolic compounds (Gülçin et al., 2019). The antioxidant activity of *Thymus* has been found to be dependent on the concentration of the extract and the type of solvent used for extraction (Kumar et al., 2018). Additionally, *Thymus* has been found to exhibit antimicrobial activity against a range of microorganisms, including bacteria, fungi, and yeast (Bicchi et al., 2013).

5 Biological Activities:

5.1 Antioxidant Activity:

Protection against the harmful effects of reactive oxygen species (ROS) is achieved through various mechanisms. These include non-enzymatic proteins, enzymes such as superoxide dismutases (SODs) and glutathione peroxidases (GPx), and dietary antioxidants like carotenoids, tocopherols (vitamin E), ascorbic acid (vitamin C), and polyphenols (Ferreira et al., 2019; Goyal et al., 2020). Non-enzymatic proteins such as transferrin, ceruloplasmin, and ferritin act as ROS scavengers. Enzymes like SODs, GPx, and catalase (CAT) are crucial in reducing oxidative stress in living organisms (Valko et al., 2005). Dietary antioxidants, including carotenoids (e.g., beta-carotene, lycopene, and lutein), tocopherols (vitamin E), ascorbic acid (vitamin C), and polyphenols (e.g., resveratrol, quercetin, and catechins), play a key role in scavenging ROS and mitigating oxidative stress-related damage (Kapoor et al., 2018; Rothwell et al., 2019). Consuming a diet rich in these antioxidants helps combat the adverse effects of oxidative stress. Antioxidants are widely used in primary and secondary prevention, with common examples being β -carotene, ascorbic acid, anthocyanins, polyphenols, and flavonoids (Bjelakovic et al., 2007). Flavonoids, in particular, can neutralize free radicals produced by the body in response to environmental stress, thereby slowing cellular aging (Karbin et al., 2015).

Material and methods

Material and methods

1 Plant material

The medicinal plant *Thymus* L. was harvested from two different regions of contrasting environmental conditions in Tiaret (Algeria) namely Rahouia (from the northern part) and Malaab (from the western mountainous part) during January 2024.

1.1 Preparation of plant powder

After the drying of aerial plant parts for one week in the darkness at room temperature, the samples were grinded using an electric grinder to obtain a fine powder. The obtained powders were put in closed bottles, labeled, and kept at room temperature until the moment of extraction (Fig. 4).

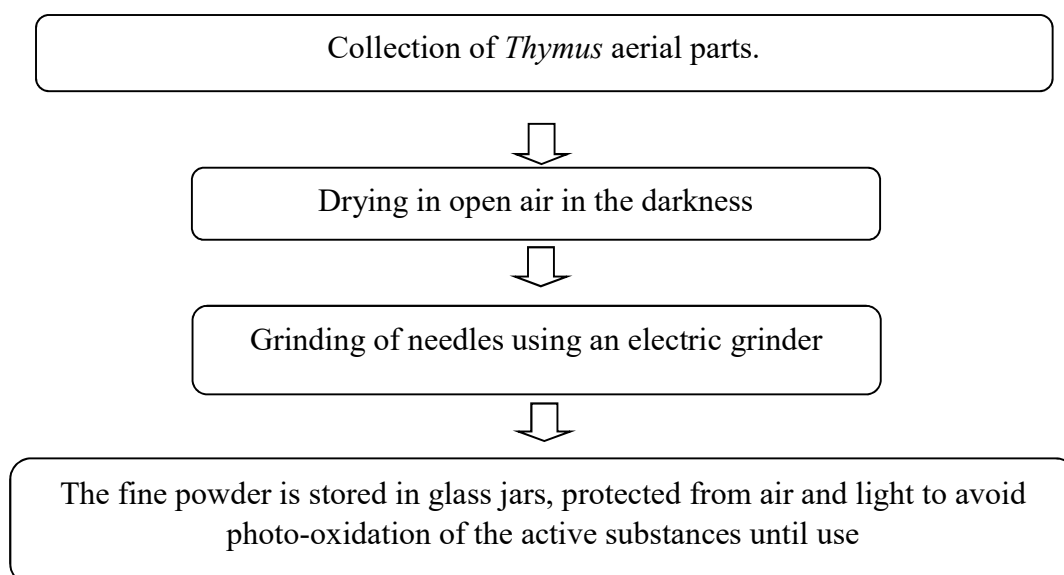


Figure 1: Preparation of the plant powder.

Material and methods

1.2 Preparation of plant extracts

Extracts were prepared by the modified method of Diallo et al. (2004).

1.2.1 Maceration

Plant extracts were prepared using 50 g of powder mixed with 500 ml of distilled water for the aqueous extract and with 500 ml ethanol 70 % for the ethanolic extract. The whole is stirred for 15 minutes in the darkness at ambient temperature then left to macerate for 24 h. The obtained mixture was filtered using Whatman paper.

1.2.2 Evaporation

- **Rotavapor processes**

- The filtrate was evaporated using a rotavapor (Fig. 6) which allows the solvent to be removed under vacuum by means of the following steps:
- Start heating the water bath. Wait until the desired temperature is reached.
- Pour the solution into the evaporating flask.
- Continue to evaporate until the solvent has completely disappeared.
- Remove the flask from the Rotavapor and allow it to cool.
- Scrape the flask with a spatula and transfer the contents to a Petri dish.
- Place the Petri dishes in the oven (40°C) until the extracts are solid.

- **The rotator constitution**

- A flask (1) containing the mixture of solvent to be evaporated
- A water bath with temperature control
- A serpentine cooler for the liquefaction of solvent vapors
- A flask (2) for the recovery of the liquid solvent (after liquefaction of the vapors)
- A motor for the rotation of the flask with the mixture to be evaporated to obtain more uniform evaporation. It is connected to a system for reducing the pressure inside the apparatus (example: a pump).
- The flask (1) contains the mixture whose solvent is to be evaporated. The solvent evaporates and the vapors produced are condensed by the refrigerant in a different container from the flask (1): the recovery flask (2).

Material and methods

2 Secondary metabolites

2.1 Polyphenols

The total phenolic content was determined by the Folin-Ciocalteu method as described by Singleton et al. (1999). The aqueous extract (1 mL) was mixed with 5 mL of Folin-Ciocalteu reagent previously diluted 10-fold with distilled water. After 1 min, 15 mL of Na₂CO₃ (20%) was added to the mixture, and the volume was adjusted to 10 mL with distilled water. The solution was kept for 2 hours of incubation in the dark at room temperature. The absorbance was then measured at 760 nm. The total polyphenol content is calculated from the calibration curve and the results are expressed as mg gallic acid equivalent (GAE)/g dry weight of the extracts.

2.2 Flavonoid

Total flavonoid content was determined following the method described by Acharya et al. (2015). Briefly, 1 mL of the aqueous extract was added to 1 mL of AlCl₃ (2%). After 15 min, the absorbance was read at 430 nm. Total flavonoid content is calculated from the calibration curve and the results are expressed as mg quercetin equivalent (QE) /g dry weight of the extracts.

2.3 Tannins

The quantification of tannins was carried out according to the method of Ouerghemmi et al. (2017). An aliquot (50 mL) of extracts was mixed with 3 mL of 4% methanol vanillin solution and 1.5 mL of H₂SO₄. After 15 min, the absorbance was measured at 500 nm. Tannin contents were expressed as mg cyanidin equivalents (CE)/ g dry weight through the calibration curve.

3 Evaluation of the biological activities of herbal tea:

3.1 Antioxidant activity using DPPH assay

The measurement of free radical scavenging activity involved assessing the reduction in absorbance of a methanol solution containing DPPH. Specifically, 200 µL of the sample was combined with 1 mL of a methanol solution containing DPPH (20 mg/L). After 30 minutes, the absorbance was determined at 517 nm. The DPPH free radical scavenging activity was determined by referencing a calibration curve, and the outcomes were expressed as milligrams of ascorbic acid equivalent (AAEq) per gram of extract (Kumar et al. 2007).

The ability to scavenge DPPH radical was calculated by the following equation:

Material and methods

$IC (\%) = [(Abs \text{ Control} - Abs \text{ Sample}) / (Abs \text{ Control})] \times 100$ Where;

- Abs Control is the absorbance of DPPH radical methanol,
- Abs Sample is the absorbance of DPPH radical + sample extract/standard.



Figure 2: preparation of ethanolic and aqueous of leaves extracts (20mg)

3.2 Evaluation of the antimicrobial activity

The antimicrobial activity of the extracts was determined by the agar diffusion method cited by (Treki et al., 2009). To prepare this medium, start by weighing 38 g of powder and combining it with 1 L of water. Thoroughly mix the ingredients to ensure homogeneity, and then heat the mixture while stirring. Bring it to a boiling point and let it boil for about one minute. Once the boiling is complete, the agar needs to be sterilized to ensure a sterile environment. This can be done by placing the agar in an autoclave and subjecting it to a sterilization process at a temperature of 121.1°C for 15 minutes.

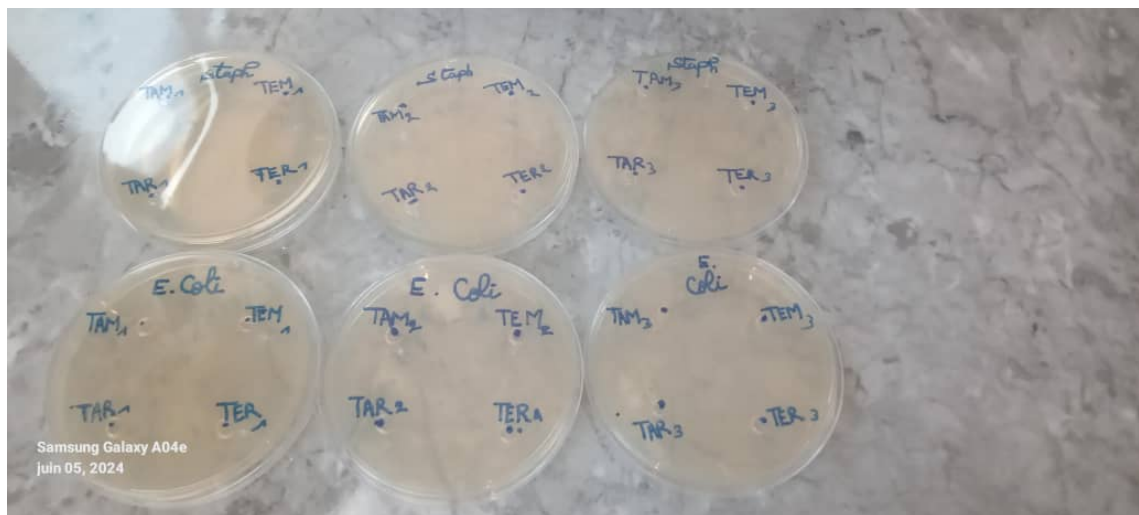


Figure 3: Bacterial suspension of E.coli and Staph

a) 6.1. Disc method

The procedure began by streaking the different bacterial strains using the streaking method. These streaked plates were then placed in an incubator at 37°C for a period of 18 to 24 hours. This incubation period allowed the growth of a young bacterial culture with isolated colonies. These isolated colonies were then used to prepare the inoculum. They were immersed in tubes containing a sterile solution of distilled water, with the aim of achieving a specific initial cell density or turbidity similar to 0.5 McFarland units. We used four strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*.

After adjusting the turbidity of the inoculum suspension, a swab was immersed in the suspension and used to streak the entire surface of the Mueller Hinton agar three times. After each swab, the Petri dish was rotated approximately 60° to ensure even distribution of the inoculum. Swabbing was also performed around the periphery of the agar surface.

To investigate the antimicrobial activity, sterile Whatman paper discs were saturated with increasing concentrations (10mg/ml, 15mg/ml, 20mg/ml) of dried extracts dissolved in dimethyl sulfoxide 70% (DMSO). These discs were then carefully placed on the surface of the Mueller Hinton agar using forceps. Following the application of the discs, the Petri dishes were incubated for a period of 24 hours at a temperature of 37°C.

Material and methods

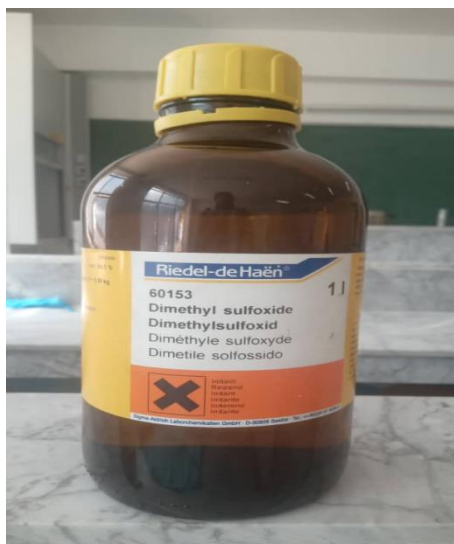


Figure 4: DMSO demethyl Sulfoxyde



Figure 5: seeding (ensemencement)

b) Wells method

In this procedure, the bacteria are introduced into agar medium that contains five wells. Among these wells, four are filled with 20 μ l of extract, while the fifth well is filled with DMSO.

Results and discussion

Results

1 Metabolit content

1.1 Polyphenol content :

The graph shows a significant positive correlation between the polyphenol content and ethanol concentration in the extracts obtained from the thyme leaves in both regions of malaab and rahouia and notably the polyphenol content is relatively low in the aqueous extract. The maximum value on the graph is 180 $\mu\text{g EAG/mg}$ ethanolic extract of malaab. The minimum value on the graph is 60 $\mu\text{g EAG/mg}$ of extract corresponds to the concentration of polyphenols in the aqueous extract.

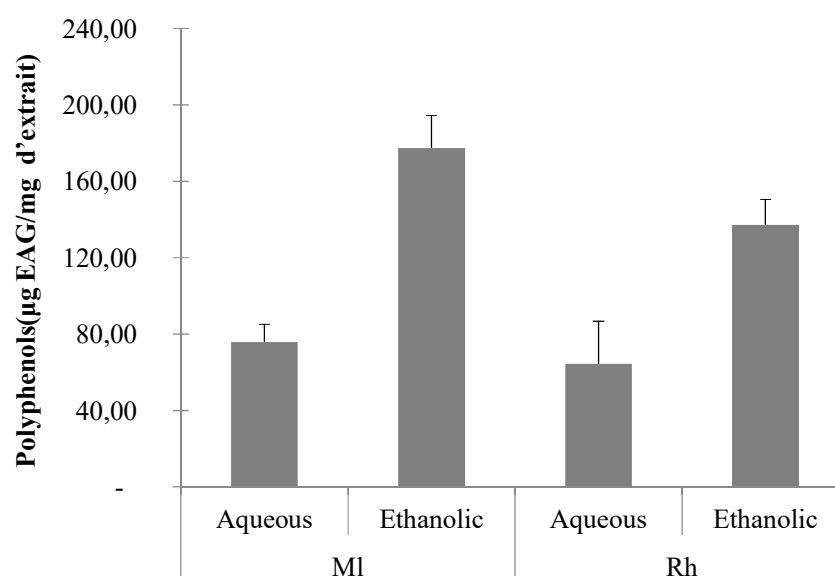


Figure 6. Variation in the polyphenol content of leaves: Levene's test for equal

1.2 Content flavonoids :

The graph illustrates a clear pattern of flavonoid content variation in the thyme leaves. The highest value in the graph is 23 EAG/mg of ethanolic extract of malaab. The lower value in the graph is 11 EAG/mg of aqueous extract of rahouia. Finally, extract aqueous of malaab is rich in flavonoids more than extract aqueous of rahouia.

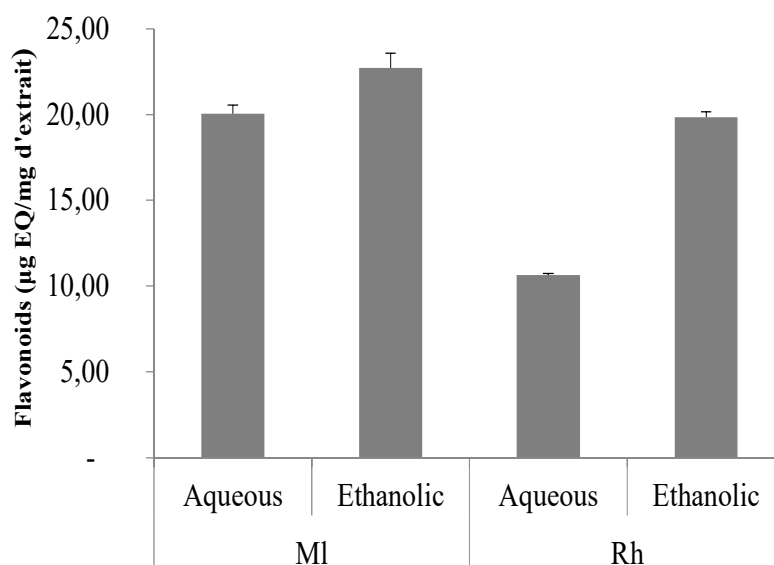


Figure 7 : Variation of flavonoid content in thyme leaves

Levene's test for equal variances of errors: $p = 0.242$ ($p > 0.05$). The difference between the sites and solvents is not significant."

1.3 Content tanins

The maximum value on the graph is $22.5\mu\text{g}/\text{mg}$ in the aqueous extract of malaab region. The minimum value is $3\mu\text{g}/\text{mg}$ of the ethanolic extract of malaab. In region of rahouia, ethanolic extract represents a high value and aqueous extract represents a low value.

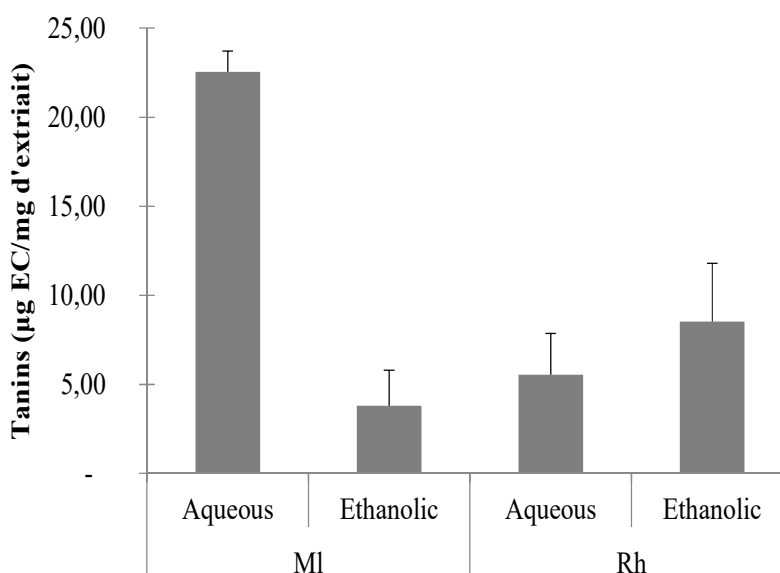


Figure 8: Content tanins

1.4 Antioxydant activity:

The maximum value of the graph it is $1,61033 \pm 0.152959$ of malaab ethanolic Then come the ethanolic extract of Rahouia with value of 1.24767 ± 0.121129 The minimum value is 0.59367 ± 0.200435 Aqueous extract Rahouia

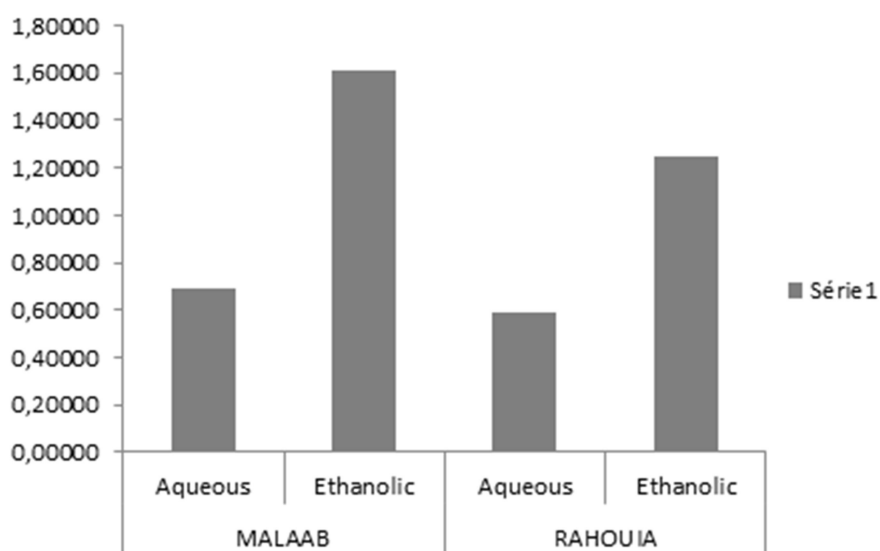


Figure 9: graphe of Antioxydant activity

1.5 antibacterial activity



Figure 10: Positif result of antimicrobial activity of thym leaves (Staph)

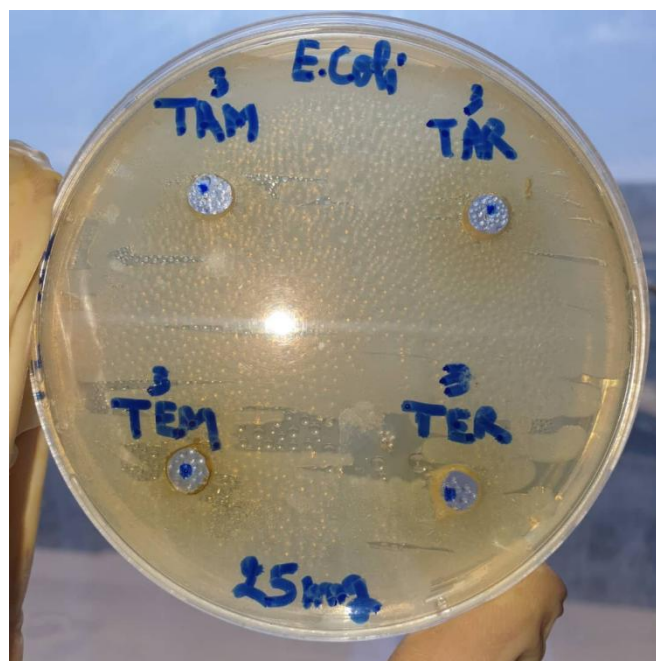


Figure 11: Negatif result of antimicrobial activity of thym (E.coli)

Results and discussion

Table. 1 Antimicrobial activity :

site	extract	Concentration	Bacteria	
RAHOUIA	aqueous		staphylococcus aureus	E. Coli
		5	No muserable	No muserable
		10	No muserable	No muserable
		15	No muserable	No muserable
		20	No muserable	No muserable
	ethanolic	5	No muserable	No muserable
		10	No muserable	No muserable
		15	No muserable	No muserable
		20	11,22 mm	11,45
MALAAB	aqueous	5	No muserable	No muserable
		10	No muserable	No muserable
		15	No muserable	No muserable
		20	12,14 mm	11,05 mm
	ethanolic	5	No muserable	No muserable
		10	No muserable	No muserable
		15	No muserable	No muserable
		20	12,15 mm	No muserable

Discussion

The current research work appraises the variation in antioxidant, antibacterial potential of thyme extracts in relation to different extraction solvents along). Maximum extract yield (21.60%) was obtained with 80% ethanol, while minimum (15.42%) for 100% ethanol.

Maximum total phenolic content (TPC), total flavonoid content (TFC), reducing power, and DPPH radical scavenging activities were exhibited by 80% methanolic extract with contribution 123.60 ± 1.51 mg/100 g, 38.11 ± 0.11 mg/100 g

The antioxidant properties of sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.), and their mixtures were examined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) scavenging activity and ferric-reducing activity of plasma (FRAP) methods.

The antimicrobial activity of the plant extracts against four bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella typhomorium*) was determined using the agar well diffusion method. Results showed the highest overall rate of increase in total phenolic (13.67 mg·GAE/g·DW), and flavonoid (6.75 mg·QUE/g·DW) contents in the thyme–sage mixture extract compared with the thyme and sage extracts

As found by GC-MS analysis of methanolic extracts, thymol, apigenin, rosmarinic acid, and carvacrol were the most abundant phenolic compounds in the thyme–sage extract. The lowest EC₅₀ (DPPH•, 55.51 $\mu\text{g}\cdot\text{ml}^{-1}$) and the highest FRAP value (95.51 mM Fe (II) mg^{-1} extract) were recorded in the extract of the thyme–sage mixture compared with sage and thyme extracts, and butylated hydroxytoluene solution (BHT).

The highest antimicrobial activity against *E. coli*, *S. aureus*, *B. cereus*, and *S. typhomorium* was observed in the thyme–sage mixture with the inhibition zone diameters of 22.13, 28.67, 31.25, and 23.65 mm, respectively. It is concluded that the extract obtained from the thyme–sage mixture has more potential to be used in the pharmaceutical and food industry as a natural antibacterial and antioxidant agent.

So The result is different from what I found,after my research work about Photochemical characterization and biological activity assessment of thyme so i was harvested from two different regions of contrasting environmental conditions in Tiaret (Algeria) namely Rahouia (from the northern part) and Malaab (from the western mountainous part) during January 2024.i prepared my Plant extracts using 50 g of powder mixed with 500 ml of distilled water for the aqueous extract and with 500 ml ethanol 70 % for the ethanolic extract. The whole is stirred for 15 minutes in the darkness at ambient temperature then left to macerate for 24 h

Results and discussion

I found that Maximum extract of malaab yield (14%) was obtained with 70% ethanol, while minimum (12,32%) of aqueous extract Ethanolic eXtract of rahouia yield(11%) was obtained also with 70% ethanol And aqueous extract yield(10,5%) while minimum.

as a comparison The extraction using 80% ethanol yielded a superior outcome compared to the extraction using 70% ethanol ,and the the ethanol extractions of bouthe region (malaab /rahouia) yield better than the aqueous extract .

Maximum total phenolic content (TPC), total flavonoid content (TFC), total taninns content (TTC) reducing power, and DPPH radical scavenging activities were exhibited by 70% ethanolic extract of malaab with contribution $75,39 \pm 2,32211 \text{ mg/100 g}$, $74,77 \pm 2,84006 \text{ mg/100 g}$ of ethanolic raouia extract

The antioxidant and their mixtures were examined using the 2,2-Dipheny Aqueous 1-1-picrylhydrazyl (DPPH•). Aqueous extract of malaab with contribution $73,84 \pm 0,79096 \text{ mg/100 g}$, aqueous extract of rahouia $59,94 \pm 1,90839 \text{ mg/100g}$.

The antimicrobial activity of the plant extracts against tow bacteria (Staphylococcus aureus and Escherichia coli.

Results showed the highest overall rate of increase in total phenolic ($180 \mu\text{g EAG/mg}$) ethanolic extract of malaab, and flavonoid (23 EAG/mg) ethanolic extract of malaab n and tannins ($22.5 \mu\text{g/mg}$) in the aqueous extracr of malaab rigion.

contents in the thymus mixture extracts of malaab compared with the thymus mixture extract of rahouia).

The highest antimicrobial activity against (E. coli, S. aureus) was observed in the thyme with the inhibition zone diameters of 11,22 mm 12,15 mm 11,91 mm in ethanolic extract and 11,45 mm 11,05mm in aqueous extract

respectively. It is concluded that the extract obtained from the extraction thyme of malaab has more potential to be used in the pharmaceutical and food industry as a natural antibacterial and antioxidant agent than the extraction thym of rahouia

and when xe compaire the inhibition zone diameters thyme of rahouia and malaab of (11,22 mm 12,15 mm 11,91 mm 11,45 mm 11,05mm) and the thyme–sage mixture with the inhibition zone diameters of(22.13, 28.67, 31.25, and 23.65 mm)

we concluded that thyme-sage has more potential and secondary metabolites to be used in the pharmaceutical and food industry as a natural antibacterial and antioxidant agent than the extracting thym of bothe rahouia and malaab

Conclusion

Conclusion

Conclusion

In conclusion, the exploration of medicinal plants, such as *Thymus* spp., continues to be a crucial endeavor in the pursuit of novel pharmaceuticals. As a rich source of bioactive compounds, these plants hold great potential for the development of future medications. The present study highlights the significant pharmacological properties of *Thymus serpyllum* extracts, including its antioxidant, anti-inflammatory, and antimicrobial activities. The presence of phenolic compounds, flavonoids, and terpenoids in varying concentrations depending on the geographic location underscores the importance of considering regional variability in the evaluation of medicinal plants.

The findings suggest that *Thymus serpyllum* can be used as a promising source of bioactive compounds for therapeutic and industrial applications. However, further research is necessary to fully characterize its potential and to isolate and identify the specific compounds responsible for its pharmacological effects. The results of this study contribute to the growing body of knowledge on the medicinal properties of *Thymus* spp. and highlight the importance of continued exploration and investigation into the vast array of medicinal plants available to us.

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