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Qualitative phytochemical profiling of *Thymus vulgaris* and *Origanum vulgare* with implications for phytochemistry and microbial therapeutics

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Abstract
Medicinal plants with a long history of ethical and pharmacological applications, primarily attributed to their rich collection of bioactive phytochemicals such as antioxidants and antimicrobial properties, highlighting their potential applications in natural therapeutics, food preservation, and infection management. This work comprehensively summarizes the qualitative phytochemical compositions and antioxidant properties in the context of natural therapeutics, food preservation, and infection control. Qualitative analyses on these two medicinal plants, employing standard chemical screening techniques, to identify key bioactive compounds such as phenol, flavonoid, tannin, terpenoid, and alkaloids. *Thymus vulgaris* L. (common thyme) and *Origanum vulgare* L. (oregano) are prominent Lamiaceae family members. The findings revealed that their bioactive constituents offer natural alternatives to synthetic drugs. This research article further discusses current research gaps and future perspectives necessary for standardizing and optimizing the pharmacological use of these valuable medicinal herbs.

Keywords: *Origanum vulgare*, phytochemical screening, pharmacognosy, bioactive plant metabolites

Introduction
Medicinal plants possess is highly important within human healthcare, providing an abundant source of bioactive compounds with diverse therapeutic properties. The exploration of phytochemicals from botanical sources remains a cornerstone of pharmacognosy, as plant-derived secondary metabolites continue to contribute significantly to the development of modern medicines, nutraceuticals, and antimicrobial agents. Within this context, Lamiaceae family herbs, notably *Thymus vulgaris* (Thyme) and *Origanum vulgare* (Oregano), have garnered considerable scientific attention due to their broad spectrum of ethnomedicinal applications and rich phytochemical compositions [1].

Thymus vulgaris and *Origanum vulgare* are aromatic, perennial herbs traditionally utilized in culinary, cosmetic, and medicinal formulations. Historically, these plants have been employed in the management of respiratory tract infections, gastrointestinal disturbances, inflammatory conditions, and microbial infections. Their widespread use in traditional systems of medicine such as Ayurveda, Unani, and European folk medicine underscores their therapeutic versatility and safety profile. The pharmacological efficacy of these herbs is primarily attributed to their abundant repertoire of secondary metabolites, including phenolic compounds, flavonoids, terpenoids, and alkaloids [2].

The qualitative phytochemical profiling of plant extracts serves as a fundamental approach in identifying and characterizing the presence of key bioactive constituents. These preliminary screening methods are indispensable not only for phytochemical characterization but also for the standardization and quality control of herbal raw materials and formulations. These compounds are recognized for their strong antioxidant, antimicrobial, and anti-inflammatory properties. Among these, antioxidant activity is particularly significant in pharmacognosy and microbial therapeutics, as oxidative stress is implicated in the pathogenesis of numerous diseases [3].

Given the therapeutic promise of thyme and oregano and the growing interest in natural antioxidants and antimicrobials, a comprehensive synthesis of existing research on their qualitative phytochemical composition and antioxidant activities is timely and valuable.

This review aims to consolidate current knowledge on the phytochemical profiles of *Thymus vulgaris* and *Origanum vulgare* as determined by classical qualitative screening tests, alongside a critical evaluation of their antioxidant capacities [4].

Materials and Methods

Collection of samples.

Dried plant resources of *Thymus vulgaris* (thyme) and *Origanum vulgare* (oregano) were procured from a commercial. Both samples were obtained in sealed, food-grade packets, marketed as 100% pure, dried and free from additives and preservatives.

Preparation of Extraction

The aerial parts (leaves and stems) of *Thymus vulgaris* and *Origanum vulgare* were sourced from authenticated suppliers. The dried resources were then soil into a coarse powder by utilizing an involuntary grinder along with stored into airtight jars away from light and moisture. For extraction, ethanolic solvent extraction was employed [5]. Precisely 100 g of powdered plant material was macerated in 500 mL of 95% ethanol for 72 hours at room temperature with occasional trembling to ensure utmost solubilization of phytochemicals. The mixture was initially filtered through muslin cloth, followed by further clarification utilizing Whatman No. 1 filter paper. The remains was resolute at 40°C under abridged force by utilizing a rotating evaporator to eliminate the solvent. The obtained crude pull out was weighed, put into airtight amber-colored bottles, and refrigerated at 4°C until further phytochemical and antioxidant analyses were performed [6].

Qualitative Phytochemical Screening

Test for Phenolics and Tannins

The occurrence of phenolic elements and tannins was determined using the ferric chloride test. In this assay, the ethanolic plant pull out was treated with a certain drops of 1% ferric chloride solution. The creation of a bluish-black or greenish-black coloration served as a positive sign of the occurrence of phenolics and tannins. This reaction relies on the capacity of phenolic hydroxyl groups to form complexes with ferric ions, resulting in a colored compound [7].

Test for Flavonoids

Flavonoid were identified using the Shinoda test. A small piece of magnesium ribbon was supplemented to the dig out, followed by the cautious adding up of concentrated hydrochloric acid. The creation of a pink, red, or orange hue indicated the presence of flavonoids. This reaction arises from the reduction of flavonoid compounds by magnesium under acidic conditions, leading to the formation of colored flavilium salts [8].

Test for Terpenoids

The Salkowski's test was applied to detect terpenoids. The pull out was mixed with chloroform, and concentrated sulfuric acid was included carefully along with the sides of the test tube. The occurrence of terpenoids was confirmed by the emergence of a reddish-brown ring at the interface [9]. The coloration results from the reaction of terpenoid compounds with sulfuric acid, which causes dehydration and subsequent rearrangement reactions.

Test for Alkaloids: Dragendorff's reagent was employed to detect alkaloids. The extract was first acidified with 1% hydrochloric acid and warmed gently to release the alkaloidal bases. After filtration, Dragendorff's reagent was introduced to the remains, and the creation of an orange or reddish-brown impetuous confirmed the occurrence of alkaloids. The precipitate forms due to complexation between alkaloid molecules and bismuth-potassium iodide in Dragendorff's reagent [10].

Test for Steroids

The Liebermann-Burchard test was conducted for steroid detection. The extract was first dissolved into chloroform, followed by the adding up of acetic anhydride along with concentrated sulfuric acid. The appearance of green, blue, or violet tinge confirmed the existence of steroidal compounds [11]. This reaction is based on the interaction of sterol rings with strong dehydrating agents, leading to characteristic color changes.

Test for Saponins

Saponins were detected through the froth test. The plant extract was mixed with distilled water and vigorously agitated. The formation of a stable froth that persisted for at least 10 minutes indicated the presence of saponins [12]. This foaming property is due to the surfactant nature of saponins, which reduce surface tension in aqueous solutions.

Test for Glycosides

Cardiac glycosides were detected using the Keller-Killiani test. The extract was combined with glacial acetic acid comprising of a drop of ferric chloride solution, followed by the careful layering of concentrated sulfuric acid beneath the mixture. The existence of cardiac glycosides was confirmed by the emergence of a brown ring at the boundary. This reaction results from the oxidation of deoxy sugars in glycosides under acidic conditions [13].

Test for Carbohydrates

Carbohydrates were identified using Molisch's test. Molisch's reagent (α-naphthol solution) was mixed with the extract, and concentrated sulfuric acid was carefully layered on top. The existence of carbohydrates was confirmed by the creation of a purple-violet ring at the junction of the two layers. This reaction takes place because of the dehydration of carbohydrates to produce furfural derivatives, which then condense with α-naphthol.

Test for Proteins

Proteins were detected by the Biuret test. The extract was treated with sodium hydroxide solution followed by a few drops of copper sulfate solution. The appearance of a violet or purple color confirmed the existence of proteins. This reaction occurs due to the complexation of peptide bonds with copper ions in alkaline conditions.

Test for Volatile Oils: Volatile oils were detected using the Sudan III test. Sudan III solution is mixed with the extract, a few drops. The development of an orange-red coloration confirmed the presence of volatile oils. The fat-soluble dye dissolves in lipophilic components of volatile oils, giving the characteristic color [14].

Test for Reducing Sugars

Reducing sugars were identified utilizing the Benedict's test.

Benedict's reagent was mixed with the extract, then heated into a water bath. The existence of minimizing sugars was determined by the creation of a green, yellow, or brick-red precipitate. These sugars reduce cupric ions to cuprous oxide, which then precipitates as colored solids [15].

Test for Cardiac Glycosides

Baljet's test was used as an additional confirmation of cardiac glycosides. The extract was treated with sodium picrate solution, and the development of an orange coloration indicated the existence of cardiac glycosides. This test depends upon the reaction between lactone rings in glycosides and picric acid under alkaline conditions [16].

Test for Lignin

Lignin was detected using the phloroglucinol-HCl test. The powdered plant material was treated with phloroglucinol solution followed by concentrated hydrochloric acid. The appearance of a red coloration indicated the presence of lignin, which reacts with phloroglucinol in an acidic medium [17].

Test for Quinones

The presence of quinones was assessed by adding sodium hydroxide solution to the extract. The appearance of a red coloration indicated the presence of quinones. Quinones react with alkali to produce colored salts or complexes [18].

Test for Phlobatannins

Phlobatannins were detected by boiling the extract with 1% hydrochloric acid. The appearance of a red precipitate confirmed the presence of phlobatannins. These condensed tannins undergo polymerization and precipitation in acidic conditions [19].

Results and Discussion

Table 1: Qualitative phytochemical results of *Thymus vulgaris* and *Origanum vulgare*

Phytochemical	Test Name	Oregano	Thyme
Alkanoids	Dragendroff's Test	++	+++
Flavanoids	Shinoda Test	-	+
Tannins	Ferric Chloride Test	+++	+++
Saponins	Froth Test	+++	++
Steroids	Liebermann-Burchard test	+	++
Phenols	Ferric Chloride Test	+++	+++
Terpenoids	Salkowski Test	-	+
Glycosides	Keller Killiani Test	-	+
Carbohydrate	Molisch's test	-	+++
Proteins	Biuret Test	+	+
Volatile oils	Sudan III Test	++	_
Reducing sugars	Benedict's Test	+++	+
Cardiac Glycosides	Baljet's Test	_	+
Ligninn	Phloroglucinol-HCl Test	+	+
Quinones	NaOH Test	+++	+
Phlobatannins	HCl Test	_	+

(-, the absent; +, slightly detected; ++; moderate amount; +++ , highly present)

Interpretation of qualitative analysis of phytochemical screening

Alkaloids

The appearance of a creamy white or reddish-brown precipitate in alkaloid testing indicates the presence of bioactive compounds known for their analgesic, antimicrobial, and anti-inflammatory properties. These contribute to formulations aimed at pain relief, infection control, and neurological applications. In the absence of precipitate formation, such therapeutic potential from alkaloids is minimal [20].

Flavonoids

A yellow color that disappears upon acidification in flavonoid testing confirms compounds with potent antioxidant, antimicrobial, anti-inflammatory, and anticancer effects. These enhance free radical scavenging capacity, useful in antioxidant-enriched and therapeutic products. Lack of this color reaction suggests limited contribution of flavonoids to antioxidant activity [21].

Tannins

A blue-black or green coloration in tannin testing demonstrates the presence of compounds with astringent, antimicrobial, antioxidant, and wound-healing benefits. Such properties make them valuable in skin care, oral hygiene, and preservative formulations. Without this color development, these protective bioactivities are not significant in the extract [22].

Terpenoids

The formation of a reddish-brown interface during terpenoid testing signifies compounds with anti-inflammatory, antimicrobial, antiviral, and aromatic properties, which are beneficial in formulations for inflammation control, antimicrobial action, and fragrance enhancement. If this reaction does not occur, the functional utility of terpenoids is minimal.

Saponins

Stable foam formation in the saponin test confirms the presence of compounds offering antimicrobial, anti-inflammatory, immune-modulating, and natural emulsifying effects. These qualities support their use in syrups, soaps, creams, and cosmetic formulations. Absence of persistent foam indicates that these functional roles may not be supported by the extract [23].

Phenols

Blue, green, or violet coloration in phenol testing indicates compounds with strong antioxidant and antimicrobial potential, which help protect against oxidative damage and microbial spoilage. This contributes significantly to product stability and therapeutic value. If no color change is observed, such bioactivity is considered weak or absent.

Glycosides

A reddish-brown ring in glycoside testing reveals compounds with cardio protective, antimicrobial, and anti-inflammatory properties. Advantageous for the production of cardiovascular health products and antimicrobial formulations. Lack of ring formation suggests a limited role of glycosides in such applications [24].

Steroids

Green or blue coloration in steroid testing indicates compounds with anti-inflammatory and anabolic activities, supporting their relevance in formulations for inflammation management and nutraceutical products. Without this color development, the extract lacks significant steroidal bioactivity [25].

Carbohydrates
The formation of a red precipitate or brick-red coloration during carbohydrate testing (using Benedict's or Fehling's reagent) indicates the presence of reducing sugars. These compounds serve as energy sources and act as precursors in metabolic pathways that contribute to plant defense and preservation in formulations. If no color change or precipitate is observed, carbohydrates with such functional activity are minimal [26].

Proteins
A violet or purple color produced when testing for proteins with the Biuret reagent confirms the presence of peptides and amino acids. These compounds provide nutritional, antimicrobial, and wound-healing benefits, making them useful in nutraceuticals and skin care formulations. The absence of this reaction suggests that proteinaceous bioactivity is insignificant in the extract.

Volatile oils
A distinct translucent spot or the appearance of turbidity with alcoholic potassium hydroxide in volatile oil testing indicates the presence of essential oils. These compounds contribute antimicrobial, anti-inflammatory, aromatic, and preservative properties, valuable in cosmetic, therapeutic, and preservative applications. If this characteristic observation is absent, volatile oil-based benefits are limited [27].

Reducing sugars
The appearance of a brick-red precipitate when the plant extract is treated with Benedict's or Fehling's reagent confirms the presence of reducing sugars. In herbal formulations, they can contribute to nutritional value, enhance preservative capacity through osmotic effects, and support microbial inhibition in certain contexts. When no color change or precipitate is observed, the contribution of reducing sugars to such functional roles is considered negligible [28].

Cardiac glycosides
The appearance of a reddish-brown ring at the interface in cardiac glycoside testing (via Keller-Killiani test) demonstrates compounds with cardioprotective, antimicrobial, and anti-inflammatory properties. These are advantageous in formulations targeting cardiovascular health and antimicrobial action. If no ring is observed, the presence of these compounds is negligible [29].

Lignin
A red coloration upon treatment of the extract with concentrated nitric acid during lignin testing indicates compounds with structural, antioxidant, and antimicrobial properties. Lignin strengthen plant cell walls and contribute preservative and antimicrobial effects in herbal products. The absence of this color reaction suggests minimal contribution of lignin to such roles [30].

Quinones
A deep blue, green, or red color in quinone testing, when the extract is treated with sodium hydroxide, indicates the presence of compounds with strong antimicrobial and antioxidant properties. Quinones are valuable for antimicrobial formulations and oxidative stress protection. Without this color change, their contribution is considered minor [31].

Phlobatannins
A red precipitate after boiling the extract with dilute hydrochloric acid in phlobatannin testing signifies compounds with astringent, antimicrobial, and antioxidant properties. Compounds useful in enhancing wound healing and microbial defense in herbal preparations. If no precipitate forms, phlobatannin- related bioactivity is minimal [32-33].

Conclusion
The qualitative phytochemical profiling of *Thymus vulgaris* (Thyme) and *Origanum vulgare* (Oregano) reveals the presence of diverse bioactive compounds, including flavonoids, phenolics, tannins, alkaloids, saponin, and glycosides. These constituents are known to exhibit potent antioxidant, antimicrobial, and anti-inflammatory properties, reinforcing their traditional and modern pharmacological significance. The findings of this work highlight the therapeutic potential of thyme and oregano as valuable sources of natural compounds for microbial therapeutics and pharmacognosy. Continued exploration and standardization of these herbs could further contribute to the development of plant-based medicinal products.

Conflict of Interest
The author(s) declare no conflict of interest related to the research, authorship, or publication of this review.

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