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Different extraction methods and antioxidant properties of thyme *(Thymus vulgaris* L.) herb

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Abstract
Medicinal plants have played an essential role in the development of human culture. Medicinal plants are a source of traditional medicine. Among different species of Thymus, *(Thymus vulgaris* L.) is used more than other species in therapeutic dosage forms. In Traditional medicine, *Thymus vulgaris* L. is cultivated in many countries by most people especially in rural areas depend on herbal medicines to treat many diseases including inflammation-related ailments such as rheumatism, muscle swelling, insect bites and pains. Also the modern medicine in essential oil of thyme has demonstrated the compounds have shown antioxidant, antibacterial and antifungal properties. Thus the highlight of the paper was to review the different extraction methods and antioxidant properties of Thyme (*Thymus Vulgaris*) herb.

Keywords: medicinal plants, traditional medicine, *Thymus vulgaris* L., antioxidants

1. Introduction

Human body constantly creates free radicals culminating in an "oxidative stress" when their elimination by antioxidant defense mechanisms is not sufficient [1]. Oxidative stress contributes to the pathogenesis of many human diseases; therefore the intake of antioxidative agents is important for the prevention of chronic diseases [2]. Antioxidants play an important role in preserving of food too. In food industry widely used synthetic antioxidants as butylated hydroxitoluen (BHT) and butylated hydroxianisole (BHA) are very effective because of their low cost, high thermal stability and efficiency but they are instable and they can play role as promoters of carcinogenesis [3, 4, 5, 6]. Due to these reasons, there is a growing interest in the study of natural additives as potential antioxidants [7]. The presence of antioxidants in many spices gives them food-preserving properties too, especially in preventing oxidation of lipids [6].

Nevertheless, the use of synthetic antioxidants in the food industry has been questioned regarding its innocuousness. Studies about spices and aromatic herbs have been widely emphasized, can act as an alternative to prevent the oxidative deterioration of food and reduce the use of synthetic antioxidants [8]. The use of natural antioxidants from food plants has the following advantages: They are accepted by the consumers; they are considered safe; they do not need safety tests; they have functional and acceptable sensory properties [9]. Studies found in literature have demonstrated that the spices belonging to the Lamiaceae family, as well as their extracts and essential oils, are efficient antioxidants [8, 10, 11, 12, 13, 14, 15].

Herbs are usually considered as plants with aromatic properties and are mainly used in spicy foods and for preparation of herbal teas in folk medicine [13]. Medicinal plants have always been considered as a source of health [16]. They are among our oldest medicines and their increasing use in recent years is evidence of public interest in alternatives to conventional medicine [11]. Since prehistoric times, herbs have also been the basis for nearly all medicinal therapy until synthetic drugs were developed in the 19th century [16].

Herbs are still found in 40% of prescription drugs [16]. In addition, herbs are used for many other purposes including beverages such as tea, dyeing, repellents, fragrances Many recent studies found out that herbs contain various phytochemicals including antioxidants [17, 18, 19, 20, 21]. At the same time it has been shown that Reactive Oxygen Species (ROS), such as hydroxyl radical, hydrogen peroxide, and superoxide anions, are produced as by-products in aerobic organisms and have been implicated in the pathology of a vast variety of human diseases including cancer, atherosclerosis, diabetic mellitus, hypertension, Aids, aging, cardiovascular diseases, cataracts, immune system decline and brain dysfunction [9]. It was also found out that free radical formation is controlled naturally by various beneficial compounds, namely antioxidants. There has been lots of evidence that consuming foods of plant origin (fruits, vegetables, tea, coffee and others) is associated with reduced incidence of these diseases [19].

Moreover, knowledge and application of such potential antioxidant activities in reducing oxidative stresses in vivo has prompted many investigators to search for potent and cost-effective antioxidants from various plant sources [19]. These research activities have contributed to new or renewed public interests worldwide in herbal medicines, health foods, and nutritional supplements. It is therefore of big interest to systematically check for the presence of antioxidants and their antioxidative capacity in medicinal herbs that are widely used in local folk medicine [22].

Studying medicinal plants helps to understand plant toxicity

and protect human and animals from natural poisons. Therefore the objective of this paper was to review the different extraction methods with different conditions used to extract thymus oil and antioxidant properties of Thyme (*Thymus Vulgaris)* herb.

2. Lamiaceae Family

Lamiaceae family is a group of about 210 genera and some 3500 species [23]. Many of them are commonly used as culinary herbs. They are often cultivated because of their aromatic qualities and also of their easy cultivation. Many species of the family are reported with high phenolic contents and antioxidant capacities [24].

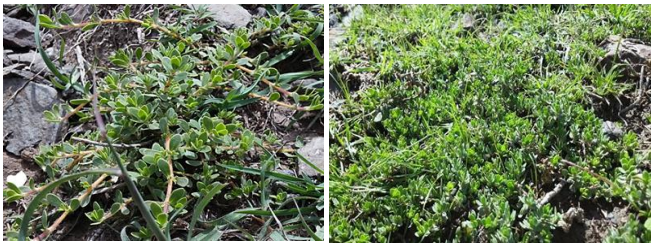
2.1 Thyme (*Thymus Vulgaris* L)

Thyme is one herb of *Lamiaceae* family. It dates back to 3500 BC by Sumerians and Egyptians [25]. Its spread to Europe was due to Romans, as they used it to give aromatic flavors to liqueurs & cheese. Thyme is the general name for the many herb varieties of the Thymus species, all of which are native to Europe and Asia. Common or garden thyme is considered the principal type, and is utilized commercially for flowering and ornamental purposes [26].

The name of Thyme in its Greek form was first given to the plant by the Greeks as a derivative of a word which meant to fumigate either because they used it as incense for its balsamic odor or because it was taken as a type of all sweet smelling herbs. Others derive the name from the Greek words *Thyo*, meaning perfume or *Thumus*, signifying courage the plant being held in ancient and medieval days to be a great source of invigoration its pleasant qualities inspiring courage [27].

Thyme is a tiny perennial shrub, with a semi ever green groundcover that seldom grows quite 40 cm tall it's each horizontal and upright habit. The stems become woody with age [28]. Thyme leaves are terribly little, usually 2.5 to 5 mm long and vary significantly in form and hair covering, depending on the variety, with every species having a rather completely different scent. *T. vulgaris* leaves are oval to rectangular in form and somewhat fleshy aerial components are used for volatile oil production, principally by steam distillation [25, 28].

India and Europe thyme grows well during a temperate to heat, dry, sunny climate, and wherever the plants don't seem to be shaded. It desires full sun to grow to its best potential. Thyme doesn't like excessive wet as a result of its condition it will gets rot diseases [29]. Thyme prefers lightweight, well-drained soils with a pH of 5.0 to 8.0. Thyme species do best in coarse, rough soils that may be unsuitable for several alternative plants [28, 30] . It is slightly spicier than oregano and sweeter than sage. Numerous cultivars and hybrids have been developed for ornamental purposes. Nomenclature can be very confusing. French, German and English varieties vary by leaf shape and color and essential oils. The many cultivars include 'Argenteus' (silver thyme).The cultivar 'Silver Queen' possess white-margined leaves [30]. In Yemen thyme is a seasonal shrub, with a green leaves that seldom grows quite 5 cm tall to extend horizontal about 50cm or more. Figure 1 shows the varieties of thyme in different cities in Yemen.



(A) **(B)**

(C)

Fig 1: (A)Thyme in Bani-Matar, Sana'a, Yemen, (B) thyme in Al-shahel, Hajah, Yemen and (C) thyme in Maswar, Amran, Yemen

Thymus vulgaris L. is one of the most popular hybrid plant used worldwide [31]. Locally *Thymus vulgaris* L species native to the Sultanate of Oman and Yemen known as "zaater" and their dried whole parts are used in herbal tea, condiments, and folk medicine. Since ancient times, this aromatic plant has been used for the preparation of different aliments to cure various curable and chronic diseases [32].

In food *Thymus vulgaris* L. can be used in fresh or dried form especially for tea. From the medical point of view, thyme has antiseptic effect due to the presence after penoidic compounds and also antifungal effects [32].

As a tea it is useful for colds, asthma and bronchitis [25] or for the treatment of acne. The antiseptic effects are mainly due to the presence of volatile oil, which mainly contains thymol, carvacrol, p-cimen, linalool, α-pinen [27] and other mono- and sesquiterpenes (β-cariophilen, germacren D or nerolidol) [33]. The antioxidant activity of wild thyme is given especially by phenolic acids (rosmarinic acid) [34] and by flavonoids (quercitin, eriocitrin, luteolin, apigenin, serpyllin) [35].

Many factors such as harvest time, seasonal variations, drying conditions, etc. may affect the composition of thyme. Thyme contains high concentrations of phenols [34]. Carvacrol and thymol are the main phenolic components which are primarily responsible for its antioxidative activity [36].

3. Methods of Extraction of Thyme Oils

Michalak [37] reported that the thyme essential oil quality and yield depend on many factors and choosing a suitable extraction method is very important.

Essential oil of thyme herb has usually been obtained by some methods such as:

3.1 Steam Distillation

Steam distillation procedure is widely used for essential oil separation; beyond its efficiency, this method gives a greater or lesser compounds instability under the influence of high temperature. There are two methods use steam distillation: simple steam distillation and Clevenger system [38].

3.2 Solvent Extraction
Solvent extraction was the main method adopted by most researchers to extract phenolics from thyme leaves. This is a process designed to separate soluble compounds by diffusion from a solid matrix using a liquid matrix. The aim of extraction is concentrate antioxidant components of raw material [37].

3.3 Supercritical Fluid Extraction
Can offer a good yield and preserve the properties of antioxidants. This method can be used for the extraction of polyphenol from plant tissue and greatly facilitates the extraction process and reduces extraction time due to low viscosity and relatively high density of supercritical fluid; it can also minimize possible degradation because it can operate in absence of light and air. Supercritical carbon dioxide is the most widely used extraction solvent [39].

3.4 Pressurized Liquid Extraction (PLE)
A relatively recent solvent extraction technique could in principle eliminate some of the drawbacks of the classical solvent extraction methods. Pressurized liquid extraction is based on the use of solvents at temperatures above their normal boiling points and pressures enough to keep the extracting fluid in the liquid state during the whole extraction process. By applying these conditions, faster extraction processes result in which typically higher extraction yields are obtained with lower volumes of organic solvents [14].

4. Antioxidant Properties
An antioxidant is a molecule that inhibits the oxidation of different molecules. Oxidation is a chemical process that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions will produce free radicals. In turn, these radicals will begin chain reactions. Once the chain reaction happens in a cell, it will cause damage or death to the cell. Anti-oxidants stop these chain reactions by removing free radical intermediates and inhibit different oxidation reactions. The leafy parts of thyme and its oil are utilized in foods for the flavor, aroma and preservation and additionally in folk medicines [25, 40]. Table 1 shows the different methods used for thyme extraction, antioxidant values and essential oil analysis. All the previous studies in Table 1 used DPPH and FRAP methods to determine the antioxidants activity.

4.1 2, 2-Diphenyl-1-Picrylhydrazyl Radical Assay (DPPH)
The DPPH assay measures the antioxidant properties of compounds in reference to their ability to scavenge the radical anion 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [20].

The DPPH is a very stable and commercially available free radical, able to accept an electron or a hydrogen atom creating the diamagnetic molecule DPPH (non radical). The radical absorbs visible light at 515 nm appearing red-violet in color, but when it is mixed with a protic solution the reduced form (non radical) is generated with the loss of the violet color and the appearance of a pale yellow color [41]. During early work on DPPH, researchers concluded that the reaction takes place through the transfer of a hydrogen atom between the radical and the solution. In fact, the reaction starts with an electron transfer, while hydrogen atom abstraction is a slow secondary reaction only occurring in strong hydrogen bond-accepting solvents such as methanol and ethanol. As in other electron transfer-based assays, the scavenging activity is strongly influenced by the pH and the solvent properties. A good choice for analyzing both lipophilic and hydrophilic antioxidants is a 50% (v/v) water/ethanol mixture [42].

The DPPH assay is a valid and easy method for evaluating the scavenging activity of antioxidant compounds because the radical is a stable molecule and does not need to be generated. The results are highly reproducible and comparable to other antioxidant detection methods [41]. A drawback is that the solvent effect has to be quantified carefully. In the case of a protic solvent, the competition of hydrogen abstraction between the antioxidant and the solvent can lead to false positive results, which obviously invalidate the determination [41].

4.2 Ferric Reducing Antioxidant Power Assay (FRAP)
The FRAP assay measures antioxidant capacity by studying the reduction of the complex ferric tripyridyl-triazine (FeIII-TPTZ) at low pH. The reduction of ferric ions to ferrous ions leads to an intense blue-colored ferrous tripyridyl-triazine complex, the formation of which can be followed spectrophotometrically [43, 44, 45]. The difference in absorbance with respect to a reaction mixture containing ferrous ions of known concentration is directly related to the total ferric reducing power of the antioxidant in the sample. The FRAP assay provides fast and reliable results for plasma, single antioxidants in pure solution, and mixtures of antioxidants in aqueous solutions. Moreover, the FRAP assay is simple and inexpensive. The only drawback of this method is that it cannot be used to determine antioxidants containing oxidizable groups [46, 47, 48].

Table 1: The different methods used for thyme extraction, antioxidant values and essential oil analysis

Country	Ratio of compounds	Main compounds	Methods of extraction	Value of DPPH	Value of FRAP	Total phenols	Essential Oil Analysis	References
Ankara, Turkey	Thymol 61% , Carvacrol, 20.6% 1.8-Cineole 14.2%, q-Cymene, 22.2% linalool 4.8% borneol, 7.5% Apinene and 6.6% Camphor	thymol and carvacrol	Solvent extraction	%92.4±0.4 IC50 =1.5 GAE (mmol/L methanol)	-	-	G.C HPLC	[30]
south of France	Thymol (41.33%), p-Cymene (18.08%), γ-Terpinene (13.12%), .1,8 Cineole (40.2%) and α-Pinene (13.2%).	thymol and carvacrol	Hydrodist-illation	IC50= 4.21 ± 0.08 µg/mL	-	-	G.C - MS	[5]

Murcia, Spain	1-Octen-3-ol 0.48% p-Cymene 1.77% 1,8 Cineole 7.23% Sabinene 1.39% Linalool 2.05% Camphor 9.55% Thymol 57.46% Carvacrol 3.44 %	thymol and carvacrol	Supercriticl fluid extraction	-	-	-	GC–MS	[39]
Morocco	Caffeic acid, ,Rosmarinic acid andQuercetin	-	Soxhlet extractor	[IC50 (mg/mL TAE)] 0.44 ± 0.02	(mmol trolox/gTAE) 65.00 ± 9.40	482.92 5.60mg equivalent affeicacid/g TAE)		[11]
Armenia	Carvacrol (21-37%) Thymol (10–17%)	Cravacrol and thymol	Solvent extraction	-	-	-	GC.MS	[24]
Palestine	-	- Cravacrol and thymol	Steam Distillation	-	-		GC-MS	[49]
Bucharest, Romania	Phellandrene2.91% α-Pinene2.79% β-Myrecene7.56% Cymene6.06% γ-Terpinene26.0% Cevacrol0.95% Thymol (30.86%) p-Cymene (30.53%) Myrcene 1.63% Sabinene 4.24% linalool 2.73% Borneol 3.16% Caryophyllene 2.48%	Cravacrol and thymol	steam distillation	89%.	-	-	GC.MS-HPLC	[50]
Sultanate of Oman	-	-	Soxhlet method-Solvent extraction	76-98%		2000 mg/L		[51]
Croatia	phydroxybenzoic acid 0.05 ±0.01 Rosmarinic acid 17.45±0.21 Apigenin-7-O-glucoside 2.37±0.01	-	Put in boiling water	69.05% IC50= 0.30g/l	-	-	HPLC-PDA	[52]
Seva Flora Valtice	-	-	Solvent extraction	-	1.13 g GAE/100 g^{-1}dw	4.16 g GAE.10 0 g^{-1}dw	-	[9]
Constantine, Algeria	-	-	Solvent extraction	70%	-	9.07 ± 0.002 mg/g TAE	-	[53]
Paris (France)	Thymol 47% p-cymene 34% Linalool1 .26% Carvacrol4 .20%g-Terpinene 2.03%a-Pinene 1.50%	Thymol and p-Cymene	Steam distillation	-	-	-	GC-MS	[54]
(Mugla, Turkey	Thymol 12–61% Carvacrol 0.4–20.6%, 1,8-Cineole (0.2–14.2%), q-Cymene (9.1–22.2%), Linalool (2.2–4.8%), Borneol (0.6–7.5%),a-Pinene (0.9–6.6%), and Camphor (0–7.3%	Thymol and Carvacrol	Solvent extraction	%92.4±0.4 IC50 =1.5 GAE (mmol/L methanol)	-	-	G.C HPLC	[55]
Iran	Thymol (41.33%), p-Cymene (18.08%), γ-Terpinene (13.12%) .1,8 Cineole (40.2%) and α-Pinene (13.2%).	Thymol and Carvacrol	Hydro-distillation	IC50= 4.21 ± 0.08 µg/mL	-	-	G.C /MS	[56]
Greece	1-Octen-3-ol 0.48% p-Cymene 1.77% 1,8 Cineole 7.23% Sabinene 1.39% Linalool 2.05% Camphor 9.55% Thymol 57.46% Carvacrol 3.44 %	Thymol and Carvacrol	Supercritical fluid extraction	-	-	-	GC–MS	[44]
Catalonia (Spain)	Caffeic acid ,Rosmarinic acid andQuercetin	-	Soxhlet extractor	[IC50(mg/mL TAE)] 0.44 ± 0.02*	(mmol trolox/gTAE) 65.00 ± 9.40	482.92 5.60mg equivalent caffeic acid/g TAE)	-	[8]
Romania (Fares)	Carvacrol (21–37%) Thymol(10–17%)	Cravacrol and Thymol	Solvent extraction	-	-	-	GC.MS	[24]
Ravenna, Italy	-	Cravacrol and Thymol	Steam Distillation	-	-	-	HS-GCMS	[57]

northern California USA	Phellandrene2.91%α-Pinene2.79%β-Myrecene7.56% m-Cymene6.06%γ-Terpinene26.0% Cevacrol0.95% thymol (30.86%) p-Cymene (30.53%) Myrcene 1.63% Sabinene 4.24% Linalool 2.73% Borneol 3.16% Caryophyllene 2.48%	Cravacrol and thymol	steam distillation	89%.	-	-	GC.MS-HPLC	[10]
Saveh,- Iran	-	-	Soxhlet and Solvent extraction	76-98%		2000 mg/L		[51]
Shambolia farm-France	Phydroxybenzoic acid 0.05 ±0.01 Rosmarinic acid 17.45±0.21 Apigenin-7-O-glucoside 2.37±0.01	-	Put in boiling water	69.05%IC50=0.30g/l	-	-	HPLC-PDA	[52]
Estahban Branch, Iran	-	-	Solvent extraction	-	1.13 g GAE.100 g^{-1}dw	4.16 g GAE.100 g^{-1}dw	-	[9]
Croatia	-	-	Solvent extraction	70%	-	9.07 ± 0.002 mg/g TAE	-	[53]
Grabowo Wielkie-Poland	-	-	Solvent extraction	-	-	517 ± 0.54 mg GAE/100 g dw	HPLC	[58]
Belgrade, Serbia	Tthymol (50.48%) p-Cymene (24.79%), Linalool (4.69%), γ Terpinene (4.14%) 1,8-Cineole (4.35%)	Thymol and p-cymene.	Solvent extraction	-	-	-	GC-MS	[59]
Dalmatia-(Croatia)	Pinene 2.0 (0.3), p-Cymen) 15.5 (0.8) Terpinene 2.2 (0.3), Linalool 40.2 (3.3), Borneol 1.2 (0.2), 1,4-Terpineol 6.8 (0.6), á-Terpineol4.1 (0.4) Linayl acetate 3.2 (0.2), Thymol 16.3 (0.6) , Carvacrol 6.8 (1.7)	Thymol and linalool	hydro-distillation	85.35%	1.3 Fe^{2+}/mmol L^{-1}	-	GC-MS	[60]
southern Morocco	-	-	Solvent extraction	92.17%	-	34.50 (µgGAE/mg DM	-	[11]

5. Conclusion

Thyme *(Thymus Vulgaris* L.) is an important medicinal plant which belongs to the Lamiaceae family. The antioxidant activity, amount of total phenolic and main components of thyme vary widely among the investigated *Thymus vulgaris* L. species around the world due to climate and method of extraction, but the most components are thymol and cravacrol in most *Thymus vulgaris* L. plant. Also this review showed that total antioxidant activity increase with increasing total phenols. There are a lot of methods for extraction of thyme oil but the most used methods are steam distillation and solvent extraction. Finally thyme has a very high antioxidant activity compared with a lot of medical plants.

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