

MOLECULAR IDENTIFICATION, PHYTOCHEMICAL CONSTITUENT DETECTION AND ANTIOXIDANT ACTIVITY OF TWO THYME (*THYMUS VULGARIS* L.) VARIETIES FROM IRAN AND SYRIA

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Abstract: The current study was conducted during (2025-2026) to determine DNA fingerprint using two molecular markers including RAPDs (Random Amplified Polymorphic DNA's and ISSR (Inter Simple Sequence Repeat) preliminary phytochemical constituent detection and antioxidant of *Thymus vulgaris* L. (Thyme) collected from Syria and Iran ethanolic extract

Using RAPD primers, all primers success in distinguishing between two examined Thyme varieties (Iranian and Syrian) by giving them a unique fingerprint. Using ISSR markers, all primers failed to give a unique fingerprint to Thyme varieties. In comparison between two markers RAPD proceeds ISSR marker in most studied criteria. Among examined phytochemical, Iranian thyme missed Trpenoid, saponin and Fuocoumarins, while Syrian Thyme missed resin, saponin and phenolate. Antioxidant activity that two plant extract possess antioxidant activity compared to control treatment. , there is increase in extract scavenging activity with increasing concentration compared to control.

Keywords: Thyme, Antioxidant, RAPD, ISSR, phytochemicals

1.Introduction

Thymus vulgaris L. (Thyme) , in Arabic it is called "Za`atar" [1] [2], belong to Labiatae (Lamiaceae) family , or the mint family which is most important and largest families and possess many classes of secondary metabolites including flavonoids , fatty derivatives , sterols , essential oils , etc. Thus ,they show an excellent antioxidant, cytotoxic, anti-inflammatory, antibacterial, antifungal, antiviral, analgesic, cardiovascular, hypoglycemic, hypolipidemic, antispasmodic, antiepileptic, anti-anxiety and anti-angiogenic activities [3].

Thyme, is initially derives from the Greek word

‘thymos’ which means courage or strength, it’s one of the most popular wild edible plants (WEPs), having many benefits, it is rich in phytonutrients, minerals, vitamins, flavonoids and antioxidants. In addition, the therapeutic effects against various diseases, this is attributed to its multi-pharmacological properties that include antioxidant, anti-inflammatory actions. Moreover, it has long been known for its antiviral, antibacterial, antifungal, and antiseptic activities [4].

Chemical constituent of medicinal plants are promising source of antibiotics with possible activity on multidrug-resistant microorganisms, the potential of plant extracts as alternative sources of novel antibiotics against pathogens [5], additionally), in the last decade, studies

on antioxidant activities of medicinal plants have increased remarkably due to increased interest of the healthy food industry in their potential of being used as a rich and natural source of antioxidants [6].

The major hurdle in potential use of herbal medicines is the lack of standardization and selection. So, it is necessary to develop the sensitive and effective skills to characterize, identify, authenticate and conserve the medicinal herbs. Proper identification and authentication of plant species is necessary to improve novel medicinal crops [7]. Typically, plants are being identified with the help of Flora of different regions based on morphological traits in the field, so, it has been difficult to distinguish the plants especially at early stage and at different geographical locations and regional distributions, to protect the patent and quality assurance of plant varieties for industries, it is necessary to develop authentic and unquestionable plant identification methods i.e. DNA fingerprinting [8] [9].

DNA-based molecular fingerprints have acted as very useful tools in various fields like classification; phylogeny, physiology, embryology, plant breeding, population mapping, ecology, genetic engineering etc. [10]. As DNA markers, RAPD (Random Amplified Polymorphic DNA) and ISSRs (Inter Simple Sequence Repeat) are simple, inexpensive, need no knowledge of the target sequence, and are easy to apply and in data analysis [11].

2.Methodology

Dried leaves of Syrian and Iranian Thyme were collected from local markets during December 2025.

Molecular identification

Dried leaves were used for genomic DNA extraction using Genomic DNA Mini Kit ,PCR amplification carried using five primers of both RAPD and ISSR ,their sequence illustrated in table 1 and amplification program shown in table 2 [12] [13].

Table 1: Primers sequence

RAPDs	Primer name	Sequence 5' → 3'
	OPA-10	GTGATCGCAG
	OPA-03	AGTCAGCCAC
	OPA-01	CAGGCCCTTC
	OPB-06	TGCTCTGCCC
	OPD-13	GGGGTGACGA
ISSRs	UBC808	AGAGAGAGAGAGAGAGC
	UBC820	GTGTGTGTGTGTGTGTC
	UBC814	CTCTCTCTCTCTCTCTA
	UBC849	GTGTGTGTGTGTGTGTTA
	UBC810	GAGAGAGAGAGAGAGAT

Table 2: PCR amplification programs.

Step	Temperature	Time
Initial denaturation	94C°	3 min
No. of Cycles = 40 Cycles		
Denaturation	94C°	1min
Annealing	Variable between 37 C° - 55 C°	1min
Extension	72C°	1min
Final extension	72C°	5min

PCR Pre Mix master mix. Bioneer Corporation Korea , (0.2ml) thin-wall 8-strip tubes with attached cup/ 96 tubes were used .primers PCR program shown in table (2).

Electrophoresis methods were applied using 1.2% agarose at 70 volts for 2 hours according to [15] using 100bp DNA ladder and ethidium bromide for agarose staining. Photographs of agarose gel electrophoresis were used to score bands, where the presence of a band was scored as '1' and absence as '0' , The polymorphism, primer efficiency, and discriminatory value were calculated for each primer using the following three equations ([16] [17]).

Phytochemical constituent

For phytochemicals constituent detection, pre prepared chemical reagent were prepared according to [18].

Antioxidant activity

The free radical scavenging capacity of the extracts was determined using DPPH(2,2-Diphenyl-1-picrylhydrazyl), The DPPH solution (0.006% w/v) was prepared in 95% methanol. Plant extracts of two plants was prepared by

five concentrations (0, 1, 0.5, 0.25, 0.125) mg/ml using methanol 95%. 100 μ l of DPPH reagent was mixed with 100 μ l of plant material samples in a 96- well microplate and was incubated at room temperature for 30min. After incubation, the absorbance was measured 517 nm using an ELISA reader, and 95% methanol was used as a control. The DPPH scavenging effect was measured using the following formula: Radical scavenging (%)= [(A) control – (A) sample\ (A) control] $\times$ 100. [19], A for absorbance.

Statistical analysis

Data for antioxidant activity was analyzed using SPSS, version 22.

3. Results and discussion

Molecular identification

Results of figures 1 and 2 show Individual RAPD and ISSR markers electrophoresis profile.

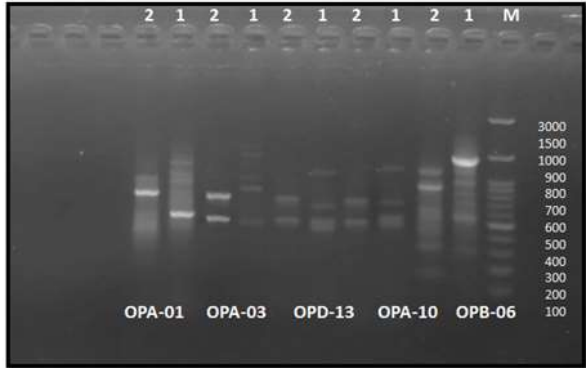


Fig.1: Amplification product of RAPD primers, M: DNA ladder for 1-Iranian *T. vulgaris* and 2- Syrian *T. vulgaris*

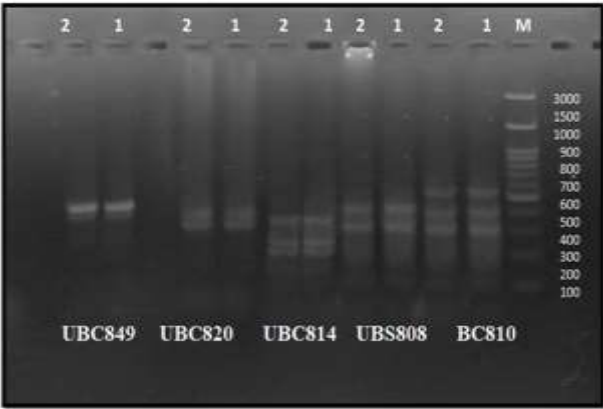


Fig.2: Amplification product of ISSR primers , M : DNA ladder for 1-Iranian *T. vulgaris* and 2- Syrian *T. vulgaris*

All primers success in giving the two examined Syria and Iranian varieties a unique fingerprint. When primer possesses an ability to produce polymorphic bands increase their ability for producing unique fingerprint [12]. [20] established that oriented breeding programs with the help of DNA fingerprinting technology will be helpful to produce distinct cultivar/genotypes with diverse genetic background and improved productivity.

Appearance of unique or polymorphic bands indicate that these cultivars possess one or more common sequence not found in others. There important arises from their ability in identification of these cultivars, thus they can be considered as genetic marker [21]. RAPD fingerprint has been can efficiently support chemo- typing of many medicinal plant species ([22], [23]. [24] Reported that polymorphic RAPD primers can successfully differentiate among different genotypes, due to different values between the amplified fragments.

The efficiency of RAPD markers were established in studying variability among *Thyme* genotypes and characterization by [25]. In comparison among two markers in table (3), RAPD markers produced highest value for (main, amplified, polymorphic bands, polymorphism, efficiency, discriminatory value and number of unique fingerprint, while ISSR marker produced highest value for monomorphic bands.

Marker	Mole cur size in bp	Main bands	Amplified Bands	Monomorphic band	Polymorphic band	Polymorphism (%)	Efficiency	Discriminatory Value (%)	No.of unique fingerprint
RA PD	14 89- 46 9	7.4	8	0.6	6.8	92.3	0.87	19.99	2
ISS R	42 5- 30 3	2.4	4.8	2.4	0	0	0	0	0

Table 3: Comparison between RAPD and ISSR markers

Since different DNA marker systems target different regions, mixed multiple marker systems should provide more complete genome information which is in agreement with the results obtained using the combined data set in this study and with several previous studies [26,27].

Variation between these markers are usually related to variation in their specificity and distribution along genome, distribution of RAPD and ISSR sequence along genome reflect in increasing number of amplified and main bands ([28].

Variation among makers results established in previous study, [29], reported that the individual marker techniques target various regions of the genome. The use of more than one marker system may provide a more informative approach while estimating the genetic diversity among the germplasm, as it might target different genomic regions. Variation among markers in detecting polymorphism and cultivar characterization established by [12, 18, 27].

Preliminary plant extract phytochemical detection

Results in table 4 showed that among examined phytochemical, Iranian thyme missed Trpenoid, saponin and Fuocoumarins, while Syrian Thyme missed resin, saponin and phenolate . Presence of many chemical in ethanolic extract supports previous results which confirmed that ethanol is more suitable solvents in extracting components of medicinal plants [30] [18] .

Presence or absent of a phytochemical in an extract solvent is related to that phytochemical solubility percentage in that solvent [31]. Plant phytochemical constituent may vary widely with respect to polarity of solvent used and consequently varying , phenolic and flavonoids concentrations and activities. Similar results reported that solvent polarity was an important factor influenced extract yield, total phenolic and flavonoid content [32].

Table (4.) Preliminary phytochemical screening in Iranian and Syrian *T. vulgaris* Ethanol extract (+) for presence and (-) for absence

Variety	Chemical reagent									
	Glycosid	Terpenes	Trpenoid	Tannins	Resins	ds	Saponins	Alkaloids	Phenolate	Fuocoum
Iranian	+	+	-	+	+	+	-	+	+	-
Syrian	+	+	+	+	-	+	-	+	-	+

3.3. Antioxidant activity

Results in figure 3 showed that there was no significant different between Iranian and Syrian Thyme extract in their antioxidant activity despite that two plant extract possess antioxidant activity compared to control (methanol treatment). Generally, plant antioxidant activity is related to extract ability in preventing an autoxidation process of other compounds or neutralize free radicals [33]. Different mechanisms suggested for antioxidant activity, it may be due to, such as prevention of chain initiation, peroxide decomposition and prevention of continuous hydrogen abstraction, free radical trapping, reduction capacity and binding of transition metal ion catalysts [34].

In recent studies by [35] and [36] established that Thyme extracts are powerful antioxidant source due to their high content of phenolic compounds ,this phytochemical presence established in table 4. Plant polyphenols have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases [37]. A study by karoui [38] established that Antioxidant activities of the thyme-enriched oil were mainly due to the presence of phenolic compounds such as thymol and hydrocarbons such as γ -terpinene and p-cymene. The thyme-enriched oil could be considered as a new and natural source of antioxidant. A study

conducted by roby [39] indicated that increasing solvent polarity positively increase its ability to dissolve phenols which consider powerful antioxidant and their results agreed with our results in producing of ethanol extract high anti-oxidant activity . Presence of phenols established in table 4, Aslan [36] proved ethanol as solvent examined for antioxidant activity due to its ability to dissolve polyphenols and flavonoids.

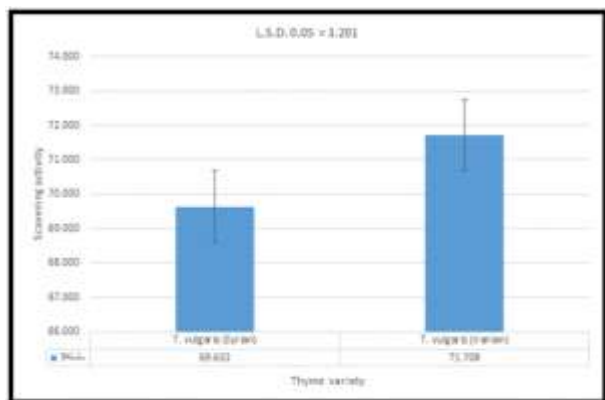


Fig 3: Effect of type of Thyme variety (Iranian or Syrian) extract in antioxidant activity using DPPH method.

In general , in figure 4 there is increase in extract scavenging activity with increasing concentration compared to control , concentrations (1, 0.5, 0.25)mg\ml gave high scavenging activity reached (90.377, 89.760, 87.149) respectively but they don't differ between each other value. Lowest value produced at concentration of 0.125 reached 84.956 but this value don't differ with values at concentration of 0.5 and 0.25 significantly.

Since phenols and flavonoids are major antioxidant constituent [40] .Phytochemicals concentrations will increase as plant extract concentration increases. previous study established that the highest concentration of phenolic compounds in the extracts especially due to using solvents of high polarity result in high contents of phenolic compounds and significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity [41]. Establishment of increasing plant extract activity with increasing concentration reported by [42] and [43].

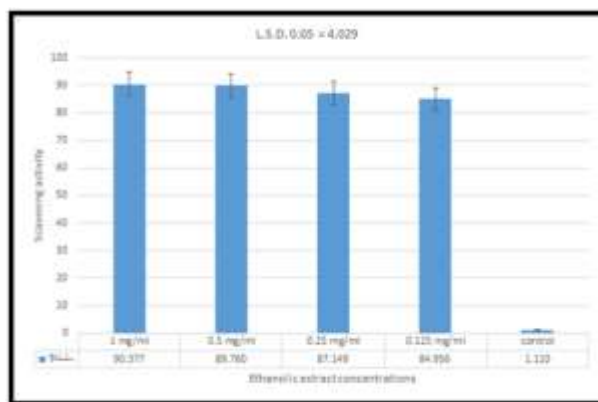


Fig 4: Effect of Syrian and Iranian extract different concentration (1, 0.5, 0.25, 0.125, control) mg/ml of on antioxidant activity using DPPH method

For interaction treatment (Thyme variety and concentration) , Results in table (5) and figure (5) showed that in general , both Iranian and Syrian extract possess antioxidant activity with increasing concentrations compared to control no significant difference observed among Syrian Thyme concentration, while significant difference observed between 1mg/ml and 0.125mg/ml in Iranian Thyme ,extract reached 91.742 and 85.546 respectively .

Thyme variety	concentration	scavenging %
(Syrian)	1 mg/ml	89.012
	0.5 mg/ml	88.886
	0.25 mg/ml	84.789
	0.125 mg/ml	84.365
	control	1.1104
(Iranian)	1 mg/ml	91.742
	0.5 mg/ml	90.634
	0.25 mg/ml	89.508
	0.125 mg/ml	85.546
	control	1.1104
L.S.D. 0.05 Interaction = 5.261		

Table 5: Effect of interaction between *T. vulgaris* variety ethanolic extract (Syrian and Iranian) at four concentrations (0,1,0.5,0.25 and 0.125) mg/ml and control on antioxidant (scavenging activity) using DPPH method



Fig 5: Configuration of the 96-well flat bottom plates antioxidant activity of Iranian and Syrian.

Varying between two plants in phytochemical types and number may attribute this variation in activity as shown in tables 4 and 7. Similar results observed by [39] in that extracts with higher antioxidant capacity also had higher polyphenol contents and concluded that the extracts obtained using higher polarity solvents were more effective radical-scavengers than were those obtained using less polar solvents, ethanol was characteristics as a solvent for phenolic compounds.

Conclusion

RAPD markers are an excellent Thyme molecular studies, ethanol is suitable for Thyme extraction since its ability in dissolving many chemicals and this reflect on two plants scavenging activity for free radicals.

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Author's Contributions

All authors had equal contributions in this project

Ethics

This study was conducted under approval by the medical ethics committee at the University of Kufa (2025). Verbal and written consent was provided by parents and agreement for publication was obtained from both participants and researchers.

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