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Total phenolic content and antioxidant activity of *Thymus vulgaris*, *Curcuma longa*, propolis and their mixtures

Original Article

Sinem Aydin
Faculty of Science and Arts, Giresun University, Department of Biology, 28200, Giresun, Turkey
sinem.aydin@giresun.edu.tr (corresponding author)

Gülşah Kadioğlu
Faculty of Science and Arts, Giresun University, Department of Biology, 28200, Giresun, Turkey

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Abstract:

The current study, we investigated phenolic content and antioxidant activity of ethyl acetate and dichloromethane extracts of thyme, turmeric, propolis and their mixtures. The highest and the lowest phenolic contents were found in ethyl acetate extract of propolis (214.94±0.023 µg GAE/mL) and dichloromethane extract of thyme (21.02±0.013 µg GAE/mL). Total antioxidant capacity of ethyl acetate extracts ranges from 127.15±0.031 µg AAE/mL and 232.2±0.028 µg AAE/mL; dichloromethane extracts ranges from 61.6±0.019 µg AAE/mL and 159.95±0.035 µg AAE/mL. CUPRAC activity and DPPH radical scavenging activity of ethyl acetate extracts are higher than dichloromethane extracts. According to the obtained results, it can be said that propolis, thyme and turmeric could be an alternative to synthetic antioxidants.

Key words:
thyme, turmeric, propolis, antioxidant activity

Apstrakt:

Ukupan sadržaj fenola i antioksidativna aktivnost *Thymus vulgaris*, *Curcuma longa*, propolisa i njihovih mešavina

U ovoj studiji, ispitivali smo sadržaj fenola i antioksidativnu aktivnost etil acetatnih i dihlormetanskih ekstrakata majčine dušice, kurkume, propolisa i njihovih mešavina. Najviši sadržaj fenola ustanovljen je u etil acetatnom ekstraktu propolisa (214.94±0.023 µg GAE/mL), a najniži u dihlormetanskom ekstraktu majčine dušice (21.02±0.013 µg GAE/mL). Ukupna antioksidativna aktivnost etil acetatnih ekstrakata kreće se u opsegu od 127.15±0.031 µg AAE/mL do 232.2±0.028 µg AAE/mL, a dihlormetanskih ekstrakata od 61.6±0.019 µg AAE/mL do 159.95±0.035 µg AAE/mL. CUPRAC aktivnost i aktivnost uklanjanja DPPH radikala viša je kod etil acetatnih ekstrakata u odnosu na dihlormetanske ekstrakte. Na osnovu dobijenih rezultata, može se zaključiti da bi propolis, majčina dušica i kurkuma mogli biti alternativa sintetičkim antioksidansima.

Ključne reči:
majčina dušica, kurkuma, propolis, antioksidativna aktivnost

Introduction

Medicinal plants are utilized worldwide for the cure of many illnesses such as asthma, gastrointestinal symptoms, skin disorders, respiratory and urinary problems and cardiovascular diseases. Plants synthesize various biologically active compounds which are crucial for them to survive in the natural environment and protect them against abiotic stresses derived from temperature, water and mineral nutrient supply (Egamberdieva et al., 2017).

Plants have been utilized as therapeutic resources such as herbal teas, crude extracts or pharmaceutical preparations (tinctures, pills and capsules) for many years. The World Health Organization (WHO) predicts that 65% of the world's population still use plants as traditional medicine (Karakaş et al., 2012).

Medicinal plants has been investigated for their antioxidant capacities by many researchers. Natural antioxidants are very effective to hinder the devastating effects caused by oxidative stress. Plants, vegetables and fruits have natural antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins. Antioxidants present in plants may protect plants from diseases (Saeed et al., 2012).

Thymus vulgaris L. (Thyme) growing wild in Turkey belongs to Labiatea family and possess many advantageous effects such as carminative, antiseptic, antioxidant and antimicrobial activities. Thymol and carvacrol are major components of thyme essential oil. Thymol and other phenolic components in *Thymus* inhibit microorganisms by increasing permeability of the cell membrane and reduction of vital intracellular substances or by disruption of bacterial enzyme systems (Tural & Turhan, 2017).

Curcuma longa L. (turmeric) belongs to Zingiberaceae family generally utilized in Indian and Chinese systems of medicine. Turmeric spice are obtained from plant rhizome known as "yellow root". It has been also utilized for the treatment of many diseases. Also, *C. longa* reduces risk of cancer (Schaffer et al., 2011)and has antiinflammatory, antioxidant and wound healing properties (Maheshvari et al., 2006).

Propolis is a natural resinous mixture produced from substances collected from some parts of plants, buds and secretions by honey bees. Propolis is one of the "natural medicines" utilized since ancient times. More than 300 active compounds were defined in propolis. Propolis has antibacterial, antiviral and antioxidant properties. Moreover, propolis is utilized in apitheraphy, cosmetic and food industry for its antioxidant and antibacterial features (Çoşkun & İnci, 2020).

In the current study, antioxidant activity and total phenolic contents of dichlorometane and ethanol extracts of thyme, turmeric, propolis and their mixtures (thyme/propolis, turmeric/propolis) at 1/1 ratio have been evaluated. We also targeted to reveal antagonistic and synergistic effects of the combination of thyme, turmeric and propolis extracts.

Materials and Methods

Providing of the samples

Thyme, turmeric and propolis were bought from a herbal shop in Giresun, Turkey.

Preparation of extracts

20 g of thyme, turmeric and propolis were extracted in a shaker for 24 h utilizing 200 mL ethyl acetate and dichloromethane, separetely. Thyme:Propolis and Curcumin:Propolis were extracted with 200 mL ethyl acetate and 200 mL dichloromethane in a shaker for 24 h, separetely. The extracts were filtered through Whatman filter paper No. 1 and residues were evaporated (40 °C) with rotary evaporator (Murugan & Parimelazhagan, 2014).

Antioxidant activity

Total phenolic content

Total phenolic contents of the extracts were determined in accordance with the method of Slinkard & Singleton (1977) utilizing gallic acid standard. Shortly, 0.1 mL extract was diluted with 4.5 mL distilled water. Then, 0.1 mL of the Folin–Ciocalteu reagent (previously diluted 3-fold with distilled water) was put into the mixture. After 3 minutes, 0.3 mL Na_2CO_3 (2%) was added. The absorbance was measured at 760 nm after incubating the mixture for 90 min. Total phenolic content of the extracts was expressed as µg gallic acid equivalents (GAE)/mL by using the calibration curve. The tests were performed in triplicate (Slimkard & Singleton, 1977).

Total antioxidant capacity

Phosphomolybdenum method was used to determine total antioxidant capacity of the extracts. 0.3 mL extract and 3000 µL reagent (contains 0.6 M sulfuric acid, 28 mM sodium phosphate and 28 M ammonium molybdate) was mixed and incubated at 95 °C for 90 min. Then, absorbance was read at 695 nm. Ascorbic acid was used as the standard (Prieto et al., 1999). The total antioxidant capacity was expressed as µg ascorbic acid equivalent (AAE)/mL. The tests were performed in triplicate.

Cupric reducing antioxidant capacity (CUPRAC) test

0.5 mL extract (250-1000 µg/mL concentration), 1.0 mL $CuCl_2$ solution ($1x10^{-2}$), 1.0 mL neocuproine solution ($7.5x10^{-3}$ M) and 1.0 mL ammonium acetate buffer (1.0 M, pH: 7.0) were mixed in a test tube. Then, the tube was vortexed and stored in a dark place for 30 min. After this period, the absorbance was read at 450 nm. Butylated hydroxytoluene (BHT) was used as a standard antioxidant agent (Özyürek et al., 2009).

DPPH radical scavenging activity

DPPH radical scavenging activity of the extracts was established by DPPH. Appropriate dilution series (250-1000 µg/mL) were prepared for ethanolic extracts in DMSO. 0.75 mL of each solution was added to 1.5 mL of a $6x10^{-5}$ M methanolic solution of DPPH. The mixture was stirred vigorously and allowed to stand in the dark at the room temperature for 30 min. Decrease in absorbance of the solution against methanol was measured at 517 nm with a Shimadzu 1240 UV-Vis spectrophotometer (Williams et al., 1995). Rutin and Butylated hydroxytoluene (BHT) were used as standard antioxidants.

The DPPH radical scavenging activity was calculated using the following equation:

DPPH Radical Scavenging Activity (%)=[(A0-A1)/A0]x100

A0: Absorbance of control
A1: Absorbance of extract or standard

Results and discussion

Total phenolic content

Phenolic compounds are significant plant metabolites which have redox properties responsible for antioxidant activity (Aryal et al., 2019). Total phenolic content was determined by utilizing the Folin–Ciocalteu reagent. The results were calculated from a calibration curve (y = 0.013x, R2 = 0.9934) of gallic acid and expressed as µg Gallic Acid Equivalent (GAE)/mL (**Tab. 1**). The highest and the lowest phenolic contents were found in ethyl acetate of propolis (214.94±0.023 µg GAE/mL) and dichloromethane extract of thyme (21.02±0.013 µg GAE/mL). Ethyl acetate extracts exhibited higher total phenolic content than dichloromethane extracts except for ethyl acetate extract of thyme/propolis. Total phenolic contents of ethyl acetate and dichloromethane extracts of thyme/propolis and turmeric/propolis were decreased when compared with ethyl acetate and dichloromethane extracts of thyme, turmeric and propolis except for dichloromethane extract of thyme/propolis.

Total phenolic content of thyme, turmeric and propolis was also searched by many authors. For example, Bulut et al. (2020) found total phenolic content of ethanol extracts of thyme leaves as 7.01±0.13 mg GAE/g (Bulut et al., 2020).

Köksal et al. (2017) determined total phenolic content of lyophilized water extract and ethanol extracts of thyme as 256 µg GAE/mg and 158 µg GAE/mg, respectively (Köksal et al., 2017). Erdoğan & Erbaş (2021) stated that total phenolic content of ethanol extract of turmeric was 82.47±2.70 mg GAE/g (Erdoğan & Erbaş, 2021). Yan & Asmar (2010) declared that total phenolic content of methanol extract of fresh and powder of turmeric was 348.±1.26 mg GAE/100 g and 2013.09±5.13 mg GAE/100 g, respectively (Yan & Asmah, 2010).

Keskin & Kolayli (2019) reported that the total phenolic substance amount of Anatolian propolis ranged between 16.13-178.34 mg GAE/g (Keskin & Kolayli, 2019). Özdal et al. (2019) reported that the total phenolic substance amount of propolis obtained from different regions of Anatolia varies between 2,748 mg GAE/100 g and 19,969 mg GAE/100 g (Özdal et al., 2019).

Collecting plants from different locations, using different extraction methods and solvents may cause discrepancy in results.

Table 1. Total phenolic contents of the extracts (µg GAE/mL)

Extract	Total antioxidant capacity
Ethyl acetate extract of thyme	73.25±0.030
Ethyl acetate extract of turmeric	175.74±0.050
Ethyl acetate extract of propolis	214.94±0.023
Ethyl acetate extract of thyme/propolis	129.2±0.007
Ethyl acetate extract of turmeric/propolis	173.61±0.008
Dichloromethane extract of thyme	21.02±0.013
Dichloromethane extract of turmeric	93.66±0.013
Dichloromethane extract of propolis	107.82±0.011
Dichloromethane extract of thyme/propolis	174.74±0.029
Dichloromethane extract of turmeric/propolis	82.89±0.025

Total antioxidant capacity

Total antioxidant capacity method is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. Ascorbic acid was utilized to compare total antioxidant capacity of the extracts (Aliyu et al., 2012). **Tab. 2** shows total antioxidant capacity of extracts. While total antioxidant capacity of ethyl acetate extracts ranges from 127.15±0.031 µg AAE/mL and 232.2±0.028 µg AAE/mL; dichloromethane extracts ranges from 61.6±0.019 028 µg AAE/mL and 159.95±0.035 µg AAE/mL. Ethyl acetate extracts exhibited higher total antioxidant capacity than dichloromethane extracts except for ethyl acetate extract of thyme/propolis. Total phenolic contents of ethyl acetate and dichloromethane extracts of thyme/propolis and turmeric/propolis were decreased when compared with ethyl acetate and dichloromethane extracts of thyme, turmeric and propolis except for dichloromethane extract of thyme/propolis. This situation might be arised by the interactions among the active substances in propolis and thyme or turmeric.

The presence of phenolic compounds could be attributable to the observed high total antioxidant capacity.

Many surveys were done by other researchers about total antioxidant capacity of propolis, thyme and turmeric. Yılmaz et al. (2017) investigated total antioxidant capacity of Propolis collected from Sakyatan (KS) and Kızılören

Table 2. Total antioxidant capacity of the extracts (µg AAE/mL)

Extract	Total antioxidant capacity
Ethyl acetate extract of thyme	127.15±0.031
Ethyl acetate extract of turmeric	177.16±0.021
Ethyl acetate extract of propolis	232.2±0.028
Ethyl acetate extract of thyme/propolis	147.52±0.031
Ethyl acetate extract of turmeric/propolis	174.6±0.046
Dichloromethane extract of thyme	61.6±0.019
Dichloromethane extract of turmeric	113.85±0.019
Dichloromethane extract of propolis	101±0.010
Dichloromethane extract of thyme/propolis	159.95±0.035
Dichloromethane extract of turmeric/propolis	85.04±0.038

(KK) regions of Konya and they found total antioxidant capacities of KS propolis and KK propolis as 2.21±0.11 mmol TEs/g extract and 2.40±0.15 mmol TEs/g extract, respectively (Yılmaz et al., 2017). Özcan & Özkan (2018) investigated total antioxidant activity of different extracts of thyme and they found that total antioxidant activity of thyme ranges from 91.14±0.87 -123.34±0.95 mg AAE/g. Bulus et al. (2017) determined that total antioxidant capacity of butanol extract of turmeric was 370 AAE/g.

Our results and literature results are different. This differences can be explained with collecting sample from different locations, using different solvents and extraction techniques.

Table 3. Cuprac activity of the extracts

Extract concentration (µg/mL)	Cuprac activity
Ethyl acetate extract of thyme	2501.4479±0.031
	5002.2108±0.0006
	7502.3390±0.020
	10002.3562±0.088
Ethyl acetate extract of turmeric	2501.7463±0.039
	5001.9936±0.003
	7502.0067±0.022
	10002.0151±0.017
Ethyl acetate extract of propolis	2501.9856±0.073
	5001.9761±0.024
	7502.0374±0.019
	10002.0573±0.109
Ethyl acetate extract of thyme/propolis	2501.801±0.043
	5001.8249±0.030
	7501.9752±0.075
	10002.0055±0.049
Ethyl acetate extract of turmeric/propolis	2501.8199±0.018
	5002.1059±0.0462
	7502.0493±0.019
	10001.9689±0.041
Dichloromethane extract of thymus	2500.5513±0.032
	5000.6634±0.049
	7501.1269±0.018
	10001.3479±0.037
Dichloromethane extract of turmeric	2501.3326±0.042
	5001.8600±0.029
	7501.8966±0.017
	10001.9426±0.055
Dichloromethane extract of propolis	2501.6693±0.059
	5001.8658±0.005
	7502.0466±0.021
	10002.1393±0.013
Dichloromethane extract of thyme/propolis	2501.8020±0.015
	5001.9255±0.011
	7501.9895±0.011
	10002.0625±0.078
Dichloromethane extract of turmeric/propolis	2500.9922±0.016
	5001.4591±0.051
	7501.7229±0.044
	10001.7569±0.007
BHT	2500.6635±0.023
	5000.7016±0.021
	7500.8283±0.024
	10000.9716±0.014

CUPRAC test

Tab. 3 presents CUPRAC activity of the extracts. Ethyl acetate extracts had better CUPRAC activity than dichloromethane extracts at 1000 µg/ml concentration. CUPRAC activity of ethyl acetate and dichloromethane extracts of thyme/propolis and turmeric/propolis were decreased when compared with ethyl acetate and dichloromethane extracts of thyme, turmeric and propolis except for dichloromethane extract of thyme/propolis at concentration of 1,000 µg/mL. This situation might be a consequence of the interactions among active substances in propolis and thyme or turmeric.

The results indicate a concentration dependent CUPRAC activity. All extracts had higher activity than BHT.

DPPH radical scavenging activity

Tab. 4 demonstrates DPPH radical scavenging potentials of extracts at different concentrations (250-1000 µg/mL) measured as a degree of discoloration displayed the extracts' scavenging potential. Dichloromethane extract of thyme showed no activity, while all the extracts exhibited lower activity than BHT and Rutin.

Table 4. DPPH radical scavenging activity of the extracts and standards (% inhibition)

Extract	Concentration (µg/mL)	DPPH radical scavenging activity
Ethyl acetate extract of thyme	250	not activity
	500	7.54±0.002
	750	39.04±0.0009
	1000	67.56±0.004
Ethyl acetate extract of turmeric	250	10.3±0.002
	500	38.53±0.0013
	750	55±0.004
	1000	75.25±0.006
Ethyl acetate extract of propolis	250	66.4±0.005
	500	67.99±0.004
	750	73.36±0.001
	1000	79.53±0.001
Ethyl acetate extract of thyme/propolis	250	45.63±0.008
	500	73.19±0.005
	750	77.57±0.005
	1000	81.64±0.003
Ethyl acetate extract of turmeric/propolis	250	38.02±0.036
	500	61.68±0.009
	750	65.23±0.008
	1000	74.16±0.004
Dichloromethane extract of thymus	250	not activity
	500	not activity
	750	not activity
	1000	not activity
Dichloromethane extract of turmeric	250	50.94±0.001
	500	55.22±0.002
	750	59.94±0.001
	1000	70.39±0.014

Extract	Concentration (µg/mL)	DPPH radical scavenging activity
Dichloromethane extract of propolis	250	26.41±0.033
	500	30.69±0.033
	750	41.67±0.023
	1000	69.08±0.005
Dichloromethane extract of thyme/propolis	250	52.9±0.066
	500	68.57±0.002
	750	70.1±0.001
	1000	74.81±0.002
Dichloromethane extract of turmeric/propolis	250	59.5±0.018
	500	69.81±0.014
	750	71.77±0.016
	1000	76.48±0.019
BHT	250	88.85±0.012
	500	89.55±0.005
	750	90.27±0.011
	1000	91.55±0.008
Rutin	250	86.80±0.008
	500	87.91±0.003
	750	90.60±0.004
	1000	91.89±0.011

DPPH radical scavenging activity of ethyl acetate and dichloromethane extracts of thyme/propolis and turmeric/propolis were increased when compared with ethyl acetate and dichloromethane extracts of thyme, turmeric and propolis at 1,000 µg/mL concentration. The best activity was detected in ethyl acetate extract of thyme/propolis (81.64%) and the worst activity was detected in ethyl acetate extract of thyme (67.56%) concentration of 1,000 µg/mL. Ethyl acetate extracts generally showed better activity than dichloromethane extracts.

DPPH radical scavenging activity was searched by many authors. Can et al. (2015) concluded that DPPH scavenging activity of propolis from Azerbaijan ranges from 15±1.00-198±3.40 (Can et al., 2015). Köksal et al. (2017) found DPPH scavenging activity (IC_{50} value) of lyophilized water extract and ethanol extract of thyme as 13.4 and 12.1, respectively (Köksal et al., 2017). Priyanka et al. (2017) investigated DPPH scavenging activity (% inhibition) of turmeric cultivars and they found that activity ranges from 49.63±2.97 to 59.58±2.95 (Priyanka et al., 2017). These differences might be a consequence of used solvent and different location of material collection.

Conclusions

The results suggest that the thyme, turmeric and propolis utilized in the current study possess antioxidant properties. Thyme, turmeric and propolis also can be used as ingredients for development of a new antioxidant agents. Further work should be focused on the isolation and elucidation of secondary metabolites in thyme, turmeric and propolis responsible for the antioxidant activity. Antioxidant activity of mixtures are lower than thyme, turmeric and propolis because of interactions of active substances in mixtures. Since the antioxidant activity of plant mixtures with propolis is lower than the antioxidant activities of these plants and propolis alone, plants and propolis should be consumed individually, not as a mixture.

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