PAPER

ANTIOXIDANT PROPERTIES, PROXIMATE ANALYSIS, PHENOLIC COMPOUNDS, ANTHELMINTIC AND CYTOTOXIC SCREENING OF *TEUCRIUM SANDRASICUM*; AN ENDEMIC PLANT FOR TURKEY

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ABSTRACT

This work was designed to evaluate the phenolic compounds and biological activities (antioxidant, cytotoxic and anthelmintic) of *Teucrium sandrasicum* extracts (ethanol and acetone) as well as to determine proximate parameters (such as proteins, carbohydrates, fat). The phenolic contents were identified using HPLC. The **e**thanol extracts exhibited higher free radical scavenging and antioxidant activities than acetone extracts. The reducing power, metal chelating and radical cation activities were found to be statistically different between the acetone and ethanol extracts. *T. sandrasicum* exhibited cytotoxic, anthelmintic activities with rich nutrient contents. Based on these results, this plant may be considered as a potentially useful source for the food and pharmaceutical industry.

Keywords: biological activities, medicinal plants, proximate, *Teucrium sandrasicum*

1. INTRODUCTION

Plants are good sources of natural functional compounds that are medically and biologically important and can be used in various fields, such as food ingredients, medicinal and pharmacological applications. For this reason, there has recently been an increase in the volume of research on the isolation and identification of these compounds. Many of these investigations especially relate to the designation of the biological activities of these compounds, such as antioxidant and cytotoxic activities (NICKAVAR and ESBATI, 2012; AL-DABBAS, 2017). The beneficial effect of medicinal plants on diseases have been revealed by a considerable number of researchers (CAKILCIOGLU and TURKOGLU, 2010). Medicinal plant species of the genus *Teucrium* have a wealth of phenolic compounds with powerful biological activities and many plants of the *Teucrium* genus have been used in the food industry, as natural preservatives and pharmaceutical applications (CANADANOVIC-BRUNET *et al*., 2006; SAROGLU *et al*., 2007; BAGCI *et al*., 2010). These plants are used to reduce inflammation and relieve indigestion and are also used as herbal medicines for coughs, asthma and stomach pain (AMIRI, 2010). In addition, *Teucrium* plants are well known for their hypoglycemic, antiseptic, antispasmodic and anthelmintic activities (GHARAIBEH *et al*., 1989; SAROGLU *et al*., 2007; REHMAN *et al*., 2016). The *Teucrium* genus is a member of the *Lamiaceae family,* of which there are more than 340 species widespread throughout the world (MAHMOUDI and NOSRATPOUR, 2013). Turkish flora includes 34 *Teucrium* species (DIRMENCI, 2012), eight of which are endemic (DAVIS, 1982). *Teucrium sandrasicum* is one of the endemic species of the *Teucrium* genus and the aerial parts of this plant are widely used in the daily diet (AKSOY-SAGIRLI *et al*., 2015). In previous limited research, several *T. sandrasicum* extracts (water, methanol, ethyl acetate, hydro-methanolic) have been evaluated for phenolic compounds, antioxidant activities and antiproliferative effects on various cell lines (AKSOY-SAGIRLI *et al*., 2015; KARAGOZ *et al*., 2015; TARHAN *et al.,* 2016). According to the literature, the biological activities of plant materials are strongly based on the nature of extracting solvents, such as polarities. Therefore, the separate examination of plant extracts, obtained from different solvents, will make a significant contribution to medicinal plant studies and their pharmaceutical applications (CANADANOVIC-BRUNET *et al*., 2006; STANKOVIC *et al*., 2011). Consequently, more research is required on the biological activities of this aromatic and medicinal plant. Within this scope, we therefore consider that *T. sandrasicum* is a plant worthy of additional investigation. Furthermore a thorough investigation of the current literature indicates that no scientific reports have been published to date concerning the antioxidant capacities, and cytotoxic or anthelmintic properties of the ethanol and acetone extracts of *T. sandrasicum*. With these points in mind, the objectives of the present study are to evaluate the antioxidant capacities, the cytotoxic, anthelmintic activities and the total phenolic and flavonoid contents of the ethanol and acetone extracts of *T. sandrasicum,* as well as the chemical composition of the ethanol extracts. In addition, the other objective of this study was to determine the proximate content of this medicinal plant.

2. MATERIAL AND METHODS

2.1. Plant materials

T. sandrasicum was collected at an altitude of 1600 m from Sandras Mountain (between Denizli-Muğla, Turkey), in July 2017. The plant material was identified by Dr. Mehmet Çiçek from Department of Biology, Faculty of Arts and Sciences, Pamukkale University,

Denizli, Turkey. A voucher specimen (*T. sandrasicum*; Herbarium No: 2017-145) has been deposited in the private herbarium of Dr. M. Çiçek (PAU) at Pamukkale University, Denizli, Turkey.

2.2. Preparation of the plant extracts

The air dried aerial parts of *T. sandrasicum* were ground to a fine powder and extracted with ethanol and acetone. Each powdered sample (30 g) were mixed with 300 mL of solvents. Extraction was carried out by shaking at 50°C for 6 h in a temperature controlled shaker and the mixture was filtered using filter paper (Whatman No.1). This procedure was repeated twice. The solvent was evaporated using a rotary evaporator (IKA RV10D, Staufen, Germany) under vacuum at 40-50°C. Samples were lyophilized (Labconco FreeZone, Kansas City, MO) and stored at -20°C until tested. All experiments were carried out in triplicates.

2.3. Chemicals

β-carotene, Linoleic acid, 2,2-Diphenyl-1-picryl hydrazyl radical (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Phosphate buffer, Iron (III) Chloride, Quercetin, Sodium phosphate, Sodium carbonate, Potassium ferrocyanide, Gallic acid, methanol, chloroform, ethanol, and acetone were purchased from Sigma-Aldrich. Butylated hydroxy toluene (BHT), Folin-Ciocalteu reagent, Tween 20 was purchased from Merck (Darmstadt, Germany). Other chemicals and solvents were of analytical grade.

2.4. Determination of phenolic compounds

2.4.1 Total phenolic content

The total phenolic content of the *T. sandrasicum* extracts was evaluated using the Folin-Ciocalteu method (SLINKARD and SINGLETON, 1977). In this method, the extract solution (1 mg/mL) was mixed with Folin-Ciocalteu reagent (1 mL) and distilled water (46 mL). After resting at room temperature for 3 min, 3mL of 2% sodium carbonate solution was added to the mixture and mixed gently. The mixture was incubated at room temperature for 2 h. Following this procedure the absorbance was confirmed as 760 nm and the outcomes were shown as mg of the Gallic acid equivalents (GAE) per gram of extract.

2.4.2 Total flavonoid content

The total flavonoid content was evaluated using to method of ARVOUET-GRAND *et al.* (1994). One milliliter solution of $AlCl_3$ in methanol (2%) was combined with the equivalent quantity of extract solution. After about 10 min the absorbance of the reaction mixtures were determined as 415 nm.. The flavonoid content was calculated from a quercetin standard curve and expressed as milligram of quercetin equivalents (QE) per gram of extract.

2.5. Determination of antioxidant activity

2.5.1 β-carotene/linoleic acid method

In this method, antioxidant capacity was determined using the method of AMIN and TAN (2002). β-carotene stock solution was prepared as follows: 2 mg β-carotene was dissolved in 10 mL chloroform. Linoleic acid (20 μL) and 200 μL of 100% Tween 20 was added for one milliliter of the solution. A rotary evaporator was used to remove the chloroform. Then the remaining residue was added to 100 mL of distilled water and the 1 mL extracts were combined with this emulsion (24 mL). A spectrophotometer was immediately used to measure the initial absorbances at 470 nm. The reaction mixture was incubated for 2 hours at 50° C. Following this, the measurement of the absorbance of this mixture was repeated, and a synthetic antioxidant (BHT) was applied as the positive control. The total antioxidant activity (AA) was calculated in following way:

$$AA=[1- (A_{samp}-A_{co})/(A^{\circ}_{samp}-A^{\circ}_{co})] \times 100$$

(A_{samp} and $A_{co:}$ absorbance at the initial time of the incubation of samples and control, respectively and A°_{samp} and A°_{co}: absorbance in the samples and control at 120 min)

2.5.2 Phosphomolybdenum method

The total antioxidant property of *T. sandrasicum* extracts was determined using the phosphomolybdenum method according to PRIETO *et al*. (1999). Various concentrations of the extracts (0.1-1.0 mg/mL) were mixed reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture (3 mL) and extracts (0.3 mL) were dispersed into test tubes and the tubes were placed at 95° C for 90 min. The absorbances of the mixtures were measured at 695 nm using a spectrophotometer. The antioxidant capacity of the extracts was expressed as μg ascorbic acid equivalents (AA) per milligram of extract.

2.6. Evaluation of radical scavenging

2.6.1 Free radical scavenging activity (DPPH)

The radical scavenging activity of the *T. sandrasicum* extracts was determined using the DPPH, as described by MERIGA *et al*. (2012) with slight modifications. Extracts of different concentrations (1 mL) were combined with 4 mL of methanolic DPPH (0.004%) solution. After vortexing the reaction mixture, the decrease in absorbance of each extract and/or control (BHT) were measured at 517 nm after 30 minutes. Results were expressed as IC_{50} (the concentration of the sample that is required to scavenge 50% of DPPH radicals).

2.6.2 ABTS radical cation scavenging activity

Experiments were performed in accordance with the method used by SHALABY and SHANAB (2013) with slight modifications. ABTS (7mM) and potassium persulphate (2.45 mM) solutions were combined and stored in a dark room for 12-16 h prior to use. Before the analysis, the ABTS solution was diluted with ethanol to an absorbance of 0.700±0.05 at 734 nm. After the addition of 4.5 mL of the ABTS reaction mixture to the various concentrations (50-400μg/mL) of the extracts (1 mg/mL), the mixture was kept at room temperature for 15 min. There was a reading of 734 nm for the absorbances of the samples.

The radical-scavenging activity of the extracts was estimated based on the ABTS color reduction, by calculating the IC_{50} (concentration in μg/mL that cause 50% inhibition of ABTS radicals).

2.7. Ferric ion reducing power activities

The method described by OYAIZU (1986) with slight modifications was used to conduct the reducing power capacity of the extracts. Different concentrations of the samples (1 mL) were combined with 0.2M phosphate buffer (1 mL) and 1% potassium ferricyanide (1 mL). The mixture was kept at 50° C for 20 min. Trichloroacetic acid (1 mL, 10%) was added to reaction mixture. The aliquot of the upper layer (1.5 mL) was combined with distilled water (1.5 mL) and ferric chloride (0.1%). After 10 min the absorbance was read, at 700 nm. The activity was expressed as mg of ascorbic acid equivalents (AA) per milliliter of extract.

2.8. Metal chelating activity

The metal chelating power of the *T. sandrasicum* extracts was determined using the method of KARPAGASUNDARI and KULOTHUNGAN (2014) with slight modifications. One milliliter of the extract and 3.2 mL of deionized water were mixed with 2 mM $FeCl_2$ (0.1 mL) solution. After 30 s, 5 mM of ferrozine (0.2 mL) was added. The reaction was activated by adding ferrozine and then the mixture was left to stand for 10 min after which the absorbances of the solutions were measured at 562 nm. The metal chelating activity was calculated in following way:

$$\text{Chelating ability } (\%) = [(A_{co} - A_{samp}) / A_{co}] \times 100,$$

(A_{co} : absorbance of the control and A_{samp} : absorbance of the extract)

2.9. Proximate analysis

The *T. sandrasicum* plant samples were analyzed to determine proteins, fat, carbohydrates, ash and energy, according to the protocols mentioned in AOAC (1995). The macro-Kjeldahl method was applied to evaluate the crude protein content of the samples. The crude fat was evaluated with a Soxhlet apparatus for which a known weight of the powdered sample was extracted with petroleum ether. The volume of ash was established by burning at 650±15°C and total carbohydrates were calculated by difference. Energy was calculated based on the following equation:

$$\text{Energy(kilocalorie)} = 4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g fat}).$$

All parameters were made in triplicate.

2.10. Quantification of phenolic compounds by HPLC

Reversed-phase high performance liquid chromatography (RP-HPLC, Shimadzu Scientific Instruments) was used for the determination of the phenolic compound. Detection and quantification was made using a diode array detector (SPD-M20A), a LC-20AT pump, a CTO-10ASVp column heater, a SIL-10ACHT auto sampler, a SCL-10Avp system controller and a DGU-14A degasser. Separations were carried out using a C-18 reversed-phase column (Agilent ZORBAX Eclipse, 250 x 4.6 mm length, 5μm particle size). The

chromatograms were examined at 278 nm. The mobile phases were A: 3.0 % formic acid in dH_2O and B: methanol. The samples were dissolved in methanol and this solution (20 μL) was injected into the column. The phenolic composition of the *T. sandrasicum* ethanolic extract was determined according to the method of CAPONIO *et al*. (1999) with slight modifications. Gallic, 3,4 dihydroxybenzoic, 4-hidroxybenzoic, 2,5 dihydroxybenzoic, chlorogenic , vanillic, caffeic, p-coumaric, ferulic, cinnamic acid and quercetin epicatechin, rutin were used as standards. The amount of individual phenolic compound was determined, based on peaks. The quantity of each phenolic compound was expressed as μg/g of the extract.

2.11. Cytotoxic activity

The potential cytotoxic capacity of the *T. sandrasicum* extracts was evaluated using the brine shrimp lethality test. (MEYER *et al*., 1982). *Artemia salina* eggs (10 mg) were incubated in 500 mL artificial seawater and under artificial light for 48 h at 28ºC. After incubation, ten nauplii were collected with a Pasteur pipette and placed into test tubes containing brine solution. In the experiments, 0.5mL of plant extract (1000, 500, 100, 50 and 10 ppm) was mixed with 4.5 mL of brine solution. The number of survivors was counted in each concentration of the extracts and the control after about 24 h. The larvae were considered dead if no movement of the appendage was observed within 10 sec. To determine the LC_{50} values, the data was analyzed using the EPA Probit Analysis Program (version 1.5) (FINNEY, 1971).

2.12. Anthelmintic activity

The anthelmintic activity of the *T. sandrasicum* extracts was determined using the methods of DASH *et al*. (2002) with slight modifications. *Tubifex tubifex* (Annelida) was used in the experiments. The average size of *Tubifex tubifex* was 1-2 cm and 6 worms were placed in a petri dish containing 20 mL test solutions of ethanol and acetone extracts. Test samples of the extracts were prepared at different concentrations (2.5, 5, 7.5, 10 mg/mL) in distilled water. Albendazole (2.5, 5, 7.5, 10 mg/mL) was used as a reference standard, while distilled water was the negative control. The worms were observed, and the time taken for paralysis and the time taken death was noted in minutes. The mean time for paralysis was logged when movement was lost, or no movement could be perceived apart from when the worm was forcefully shaken. The time of death was recorded of each worm after ascertaining that the worm failed to move when shaken or when given external stimuli.

2.13. Statistical analysis

All analyses were performed in triplicate and the results presented as mean±SE (Standard Error) and the results analyzed using the MINITAB Statistical Package program. To see how the groups differed from each other, the variations between the different extracts were tested with Analysis of Variance (ANOVA) and Tukey (P<0.05), and the different groups were shown with different letters in the same column. If there were only two groups then a t-test was used.

3. RESULTS AND DISCUSSION

Antioxidant activity determination methods depend upon various parameters such as the concentration and the structure of the compound to be analyzed. For this reason, there is

no standard method for determining the antioxidant activity of a compound and one single method cannot fully describe the antioxidant activity (DU *et al*., 2009; JABRI-KAROUI *et al*., 2012). Consequently, we used six complementary antioxidant methods (radical scavenging (DPPH and ABTS), total antioxidant (β-carotene /Linoleic acid and phosphomolybdenum), metal chelating and reducing power activities) to evaluate the true antioxidant potential of the extracts.

3.1. Total phenolic and flavonoid content

The total phenolic and flavonoid contents in the ethanol and acetone extracts from *T. sandrasicum* have been determined in the present study. Our results revealed that in the ethanol extract, the total phenolic content with 107.94±0.59 mgGAE/g was higher than that of the acetone extracts with 78.2±1.5 mgGAE/g and this was found to be statistically different ($t=18.21$, $df=10$, $p<0.001$). The phenolic content for the acetone extract determined in the present study was lower than that determined by STANKOVIC *et al.* (2010) (acetone extract of *T. chamaedrys*). In addition, the variable amounts of total phenolic content in the different extracts may be due to solvent polarity (MARINOVA and YANISHLIEVA, 1997).
The total flavonoid content was found 65.96±0.19 and 51.61±0.56 mgQEs/g in acetone and ethanol extracts respectively and these were statistically different ($t=24.30$, $df=9$, $p<0.001$). These results obtained are in line with those of TARHAN *et al.* (2016) who found total flavonoid content varied from 30.23-95.12 mg/g in ethyl acetate, water and hydromethanolic extracts from *T. sandrasicum*. In addition, similar to our study, BAKARI *et al*. (2015) found acetone extract to have a higher total flavonoid content than the ethanol extract in *T. polium*.

3.2. Antioxidant activities

3.2.1 Total antioxidant activity (β-Carotene-linoleic acid and Phosphomolybdenum methods)

β-carotene/linoleic acid is used to measure antioxidant activity. Antioxidants minimize the oxidation of lipid components in cell membranes, or inhibit the conjugated diene hydroperoxides, known to be carcinogenic, generating from linoleic acid oxidation (TEPE *et al*., 2007). In this study, the antioxidant activity of the ethanol extract from *T. sandrasicum* (80.18±1.34%) was better than the acetone (73.61±0.95 %) extract (Fig. 1).
These results are in line with those of BAKARI *et al.* (2015) who found ethanol extract exhibited higher antioxidant properties than the acetone extract in *T. polium*. In addition, total antioxidant activity for the ethanol and acetone extracts determined in this study were higher than those reported by BAKARI *et al*. (2015) (*T. polium*). In this study, there were statistically differences among the antioxidant contents of the different extracts of T. *sandrasicum* and BHT ($F_{2,24}= 109.76$, $p<0.001$) (Fig. 1). Although the synthetic antioxidant (BHT) showed the highest antioxidant activity (over 90 %), the ethanol and acetone extracts were as effective as standard and they seemed to reduce the oxidation of linoleic acid, a key concern for the food industry.
The antioxidant activity of the samples was also evaluated using the phosphomolybdenum assay, according to the method of PRIETO *et al.* (1999). Similar to the β-Carotene-linoleic acid test system, in this method the ethanol extract from *T. sandrasicum* showed stronger antioxidant capacities than the acetone extract (Table 1).

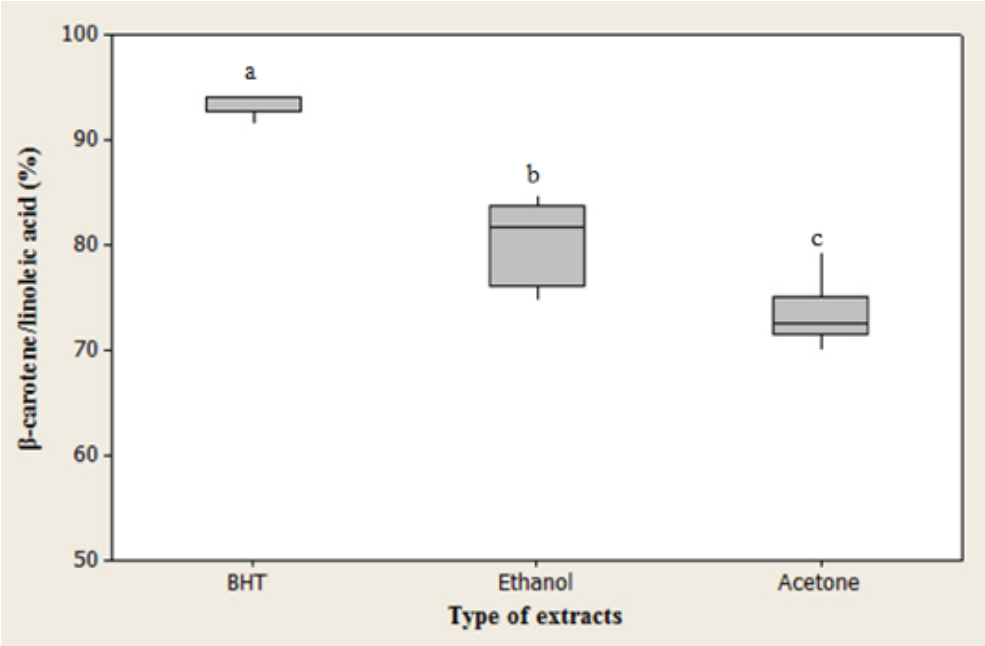

Figure 1. Antioxidant activity of *T. sandrasicum extracts.*
Ethanol extract of *T. sandrasicum*; Acetone extract of *T. sandrasicum,* BHT: Standard antioxidant.
(different groups were shown with different letters on each boxplot)

Table 1. Antioxidant properties of *T sandrasicum* extracts.

Sample	DPPH (IC_{50}, $\mu g\ ml^{-1}$)*	ABTS (IC_{50}, $\mu g\ ml^{-1}$)*	Phosphomolybdenum ($\mu g/mg$)*	Power reducing (mg/mL)*
Ethanol	122.60±1.35 b	174.86±1.52 a	104.03±3.3 a	0.32±0.01 a
Acetone	184.76±6.07 a	119.11±6.22 b	74.7±6 b	0.24±0.03 b
BHT	31.64±1.52 c	12.89±1.20 c	nt	nt

BHT: Standard antioxidant, nt: not tested.
*Values are mean of three replicate determinations (n=3)±standard error. Mean values followed by different letters in a column are significantly different ($p<0.05$).

The antioxidant activities were found to be statistically different between the ethanol and acetone extracts ($t=4.31$, $df=12$, $p<0.001$). As previously reported by NICKAVAR and ESBATI (2012) and CAKIR *et al.* (2003), our results also showed that the high antioxidant capacities of the ethanol extract of *T. sandrasicum* is due to the presence of high phenolic content.

3.3. Radical scavenging activity (DPPH and ABTS)

A stable free radical of a deep shade of purple on scavenging DPPH becomes yellow. The level of yellowing indicates the scavenging potential of the extracts, in terms of hydrogen donating ability. Consequently, DPPH is generally used as a substrate to ascertain antioxidant capacity (DUH *et al.*, 1999). The results of the DPPH in present study are given in Table 1. The higher DPPH radical scavenging activities were associated with the lower IC_{50} values. The ethanol extract exhibited higher scavenging activity than the acetone extract and there were statistically differences among the radical scavenging activities of

the different extracts of *T. sandrasicum* and BHT ($F_{2,24}$= 434.32, p<0.001). The present study has demonstrated that the ethanol and acetone extracts from *T. sandrasicum* have a radical scavenging capacity and the key role of the phenolic content as scavengers of free radicals has been emphasized in several other reports (KOMALI *et al*., 1999). When the acetone extract of *T. sandrasicum* was compared with *T. montanum* (STANKOVIC et al., 2011) and *T. polium* (BAKARI et al., 2015) of which the DPPH free radical scavenging activities were found to be 108.10 μg/mL and 13 μg/mL respectively, the DPPH free radical scavenging activity of the acetone extract of *T. sandrasicum* was lower than those of these species.
The ABTS scavenging capacity of plant extracts from *T. sandrasicum* were determined and the results are presented in Table 1. The values of IC_{50} were in the following order: BHT < acetone extracts< ethanol extracts. AKSOY-SAGIRLI *et al*., (2015) used ABTS for the determination of radical scavenging activity in methanol extracts from *T. sandrasicum.* In the present study we also used ABTS to investigate scavenging activity in ethanol and acetone extracts from *T. sandrasicum* and found that the ethanol and acetone extracts of *T. sandrasicum* have radical scavenging activity. Free radicals, which are produced in the human body by chemicals or metabolic processes are capable of oxidizing biomolecules (HALLIWELL and GUTTERIDGE, 1989) and exposure to free radicals causes cell damage, which may increase the risk of various diseases, such as cancer, heart diseases and diabetes. As with antioxidants, by inhibiting the formation of free radicals, free radical scavengers naturally protect cells from the damage caused by harmful molecules (PERCIVAL, 1998). The present study reveals that *T. sandrasicum* extracts could serve as free radical scavengers and due to these properties, they may be used as an ingredient in food.

3.4. Ferric ion reducing power activities

The reducing ability describes how easily one substance can give electrons to another. A powerful reducing agent is inclined to give electrons. The reducing power method measures the ability of components that act as antioxidants to reduce ferric ion (SINGH *et al*., 2012). In the present study, the reducing ability of ethanol and acetone extracts from *T. sandrasicum* were measured, and the results of this activity demonstrated that the ethanol extract showed a higher reduction ability than those of the acetone extracts (Table 1). The reducing power activities were found to be statistically different between the ethanol and acetone extracts (t=2.34, df=9, p<0.05). According to these results, the ethanol and acetone extracts of *T. sandrasicum* possess antioxidant capacity. This is because the reducing capacity of a compound serves as potential antioxidant activity (SINGH *et al*., 2012). In addition, the reducing power of ethanol extract may be due to the high level of phenolic content, which acts as an electron donor. Similarly, numerous studies advocate an association between the reducing power and the total phenolic content (GONCALVES *et al*., 2013).

3.5. Metal chelating properties

The metal chelating ability of the studied *T. sandrasicum* extracts were determined by measuring the iron-ferrozine complex. The metal chelating property of the ethanol and acetone extracts from *T. sandrasicum* were evaluated and these results showed that the ethanol extract (55.85±4.22 %) exhibited better metal chelating activity when compared with the acetone extract (26.67±1.48 %). Although the synthetic metal chelator (EDTA) exhibited the highest chelating activity (over 80 %), the ethanol and acetone extracts inhibited complex of ferrous, ferrozine and this revealed that they exhibit chelating activity (Fig. 2). The metal chelating capacity is important, because this activity reduces the

amount of catalyzing transition metal in lipid peroxidation (DUH *et al.*, 1999). For this reason, the presence of the chelating properties of the extracts contribute directly to their antioxidant properties.

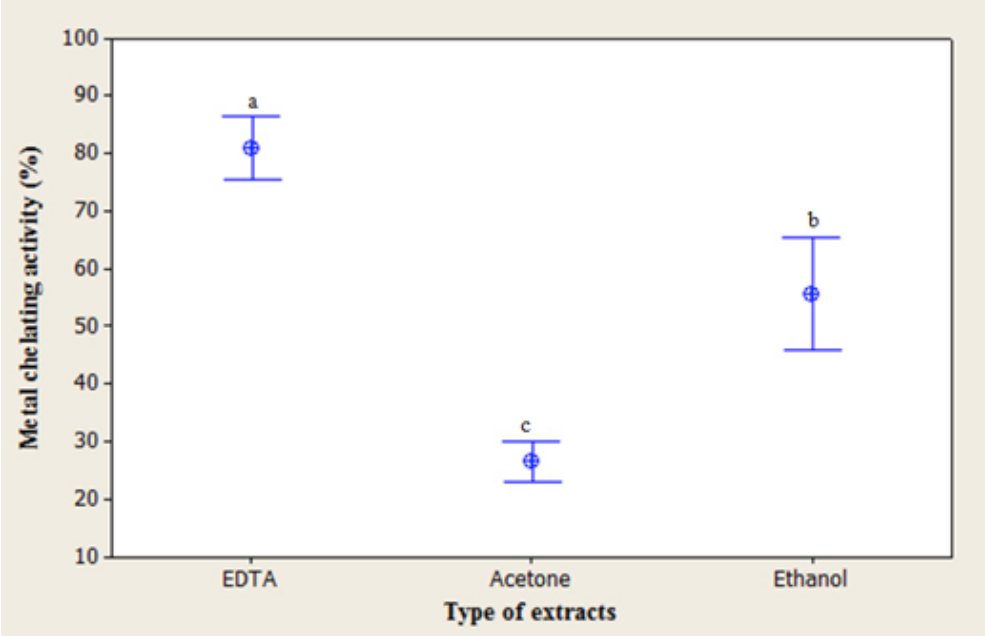

Figure 2. Metal chelating activity of *T. sandrasicum extracts.*
Ethanol extract of *T. sandrasicum*; Acetone extract of *T. sandrasicum*, EDTA: Standard antioxidant.
(different groups were shown with different letters on each bar)

3.6. Proximate analysis

The evaluation, determining the moisture, crude protein, crude fat, ash, carbohydrate, fibre and energy, as a proximate analysis of the aerial parts of *T. sandrasicum*, is presented in Table 2. When compared with earlier studies, the protein content of *T. sandrasicum* was found to be lower than those of *T. muscatense* (REHMAN *et al.*, 2016) and *T. polium* (HUSSAIN *et al.*, 2013). In contrast with the study of REHMAN *et al.* (2016), the carbohydrate content of *T. sandrasicum* was found to be lower than *T. muscatense*. The fat content and the energy value of the *T. sandrasicum* were lower than the fat content and the energy value of the *T. polium* (HUSSAIN *et al.*, 2013).

Table 2. Proximate analysis of *T. sandrasicum.*

Constituents	Aerial parts
Ash (g/100 g dw)	4.76±0.52
Carbohydrate (g/100 g dw)	17.06±1.28
Proteins (g/100 g dw)	2.43±0.10
Fat (g/100 g dw)	1.10±0.12
Moisture (g/100 g fw)	42.17±0.9
Fibre (g/100 g dw)	28.48±0.1
Energy (kcal/100 g dw)	87.86

The plants species, especially medicinal plants are also used as food or a food supplement and evaluating their nutritional contents can help to understand the significance of these plant species as a dietary supplement and for pharmaceutical applications. Proximate analysis of this plant plays a decisive role in assessing its nutritional significance and revealed that this species is good source of nutrients as well as can contribute towards nutritional requirements (PANDEY *et al.*, 2006; ADNAN *et al.*, 2010).

3.7. Phenolic composition

It has been established that the Lamiaceae species comprise a range of secondary metabolites, including phenolic acids and flavonoids. In present study, phenolic compositions of ethanol extract of *T. sandrasicum* were identified using HPLC method. Phenolic compound that were determined in ethanol extract are listed in Table 3 and the main phenolics were identified as caffeic acid and rutin (Fig. 3).

Table 3. Phenolic components in the ethanol extract of *T. sandrasicum*.

No	Phenolic component	Approximate Rt (min)	μg/g*
1	Gallic acid	6.8	917.35±0.08
2	3,4 dihydroxybenzoic acid	10.7	92.47±0.01
3	4-hydroxybenzoic acid	15.7	1066.40±0.08
4	2,5 dihydroxybenzoic acid	17.2	61.06±0.02
5	Chlorogenic acid	18.2	462.02±0.05
6	Vanilic acid	19.2	563.29±0.09
7	Epicatechin	21.3	1648.12±1.02
8	Caffeic acid	22.7	22727.28±5.06
9	p-Coumaric acid	26.1	1.47±0.05
10	Ferulic acid	30.1	72.06±0.01
11	Rutin	45.6	3392.28±1.06
12	Cinnamic acid	71.1	315.40±0.03
13	Quercetin	70.4	2893.08±0.02

*based on dry weights

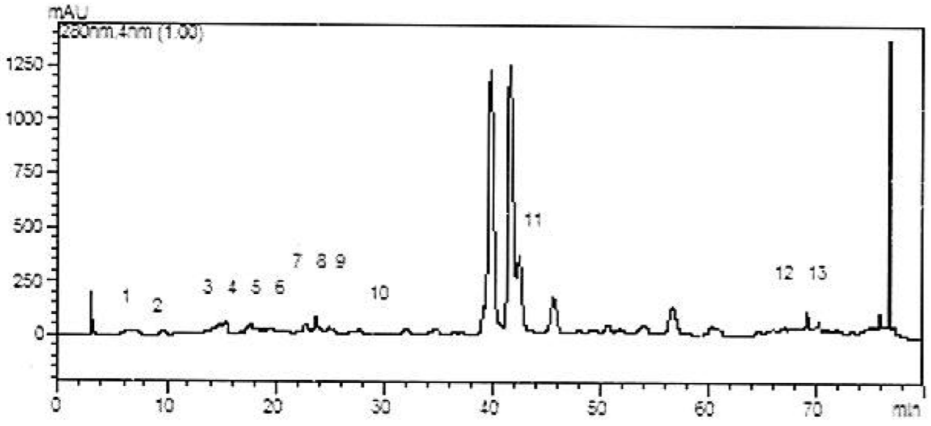

Figure 3. HPLC chromatograms of phenolic components in the ethanol extracts of *T. sandrasicum*.

Some phenolic compounds determined in present study, such as ferulic, gallic, caffeic, vanillic, chlorogenic acids and rutin, in previous studies were obtained from Lamiaceae plants (CANADANOVICH-BRUNET *et al*., 2006; ROBY *et al*., 2013; KASKA *et al.*, 2018). In addition, previously studies have shown that, from these phenolics, caffeic acid exhibits anticarcinogenic properties that act as a carcinogenic inhibitor (MAGNANI *et al*., 2014). In brief, nowadays the identification and measurement of plants phenolic compounds are considered to be effective mechanisms for ascertaining the importance of plants for human health (AMAROWICZ *et al*., 2010). This is because phenolic content can directly contribute to the antioxidant capacity of the plants (DUH *et al.*, 1999).

3.8. Cytotoxic activity

The brine shrimp cytotoxicity test is a practical and economic method for the investigation and assessment of toxicity, antifungal and pesticidal effects of plants. LC_{50} values of less than 1000 μg/mL are regarded as bioactive when using the brine shrimp lethality test to calculate the toxicity of plant extracts (MEYER *et al*., 1982). The lethality of ethanol and acetone extracts were 389.661 and 658.032 μg/mL respectively, and the extracts possessed high cytotoxic activities against brine shrimp. The lethality of these extracts from *T. sandrasicum* indicates the presence in this species of potent cytotoxic components, which require further investigation. The present study suggests the need for further investigations of this plant, in order to ascertain the potential cytotoxic compound.

3.9. Anthelmintic activity

In the present study, an investigation was made of the anthelmintic activities of *T. sandrasicum* extracts (ethanol and acetone). The results presented in Table 4 show that the ethanol and acetone extracts obtained from *T. sandrasicum* is active against *Tubifex tubifex*.

Table 4. *In vitro* anthelmintic activity of *T. sandrasicum*.

Type of extract	Concentration used (mg/mL)	Time (min) taken for paralysis (X±S.E.)*	Time (min) taken for death (X±S.E.)*
Control (Distilled water)	-	-	-
Ethanol	2.5	37.33±1.58 a	48.5±1.63 a
	5	21±1.93 b	31.67±1.38 b
	7.5	15.67±1.20 bc	21.67±0.62 c
	10	10.17±0.75 c	14±0.82 d
Acetone	2.5	22.33±1.52 a	31.5±1.43 a
	5	17.83±0.54 b	21±0.48 b
	7.5	12±0.45 c	16±0.45 c
	10	9±0.37 c	12.65±0.56 d
Albendazole (Standard)	2.5	52.33±2.73 a	61±3.33 a
	5	33±1.48 b	48.5±3.48 b
	7.5	21.17±2.01 c	35.5±3.21 c
	10	13±0.68 d	20.5±1.57 d

Values are mean±S.E. of six worms.
*Mean values followed by different letters in a column are significantly different ($p<0.05$).

All doses of the *T. sandrasicum* extracts (ethanol and acetone) showed better anthelmintic activity, in terms of promoting paralysis and causing death, than the standard. The strong anthelmintic activity of *T. sandrasicum* extracts may be due to the existence of rich polyphenolic compounds. Both humans and animals have benefitted from a broad range of medicinal plants in the treatment of parasitic infections in. Anthelmintics, derived from plant sources, present some advantages, such as pharmacological effectiveness and lower toxicity for animals and humans (PEIXOTO *et al*., 2013). Nowadays, there is an increasing interest in studies on the screening of new and effective medicinal plants that have anthelmintic properties. *T. sandrasicum* extracts possess wormicidal activity and could be effective against the parasitic infection of humans and animals. Hence, the lethality of the potent anthelmintic components in this species requires further investigation.

4. CONCLUSIONS

The results revealed in the present study have shown that the acetone and ethanol extracts from this plant have strong antioxidant properties. They have also shown that the plant possesses rich phenolic and flavonoid compounds, making it a good source of nutrients. Furthermore, *T. sandrasicum* extracts have been shown to have cytotoxic and anthelmintic activities. The present study therefore suggests that this plant could be considered as a source of natural agents in the food industry and can be used as a new anthelmintic and cytotoxic agent for pharmacological applications. Further investigation is required to isolate and identify the antioxidant, anthelmintic and cytotoxic components found in this plant. This will in turn increase information on the usability of the plant for the food industry and pharmacological applications.

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