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ELUCIDATION OF NUTRITIONAL, PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES OF TEUCRIUM POLIUM L GROWN IN LIBYA

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ABSTRACT

Consumption of plant compounds play a crucial role in promoting health by improving the nutritional value of diet and preventing several chronic conditions such as cancer, cardiovascular, diabetes and neurological diseases. The current work aimed to analyse qualitatively and quantitatively the stem and leave extracts of Teucrium *polium L. (Lamiaceae*) grown in Libya for proximate, nutritional and phytochemical constituents as well as their biological properties. Methanolic extracts of leaves and stems were employed to evaluate their content of some of the primary and secondary compounds by several standard methods using a spectrophotometer. Additionally, the antioxidant properties of extracts were assessed using various in vitro systems including 1,1- diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO) radicals scavenging ability, Lipid peroxidation activity, phosphomolybdenum reduction, and reducing power activity at different concentrations (6.25 to 100 mg/ml). Moreover, the cytotoxic effect of extracts was tested by measuring the Hemolytic activity against human red blood cells (hRBCs) while the anti-inflammatory was investigated by measuring the Inhibition of albumin denaturation. Results showed that the leaves had a higher concentration of carbohydrates, protein, fat, phenols, flavonoids, flavanols, tannins, catechins and coumarins, whereas the stems had a higher concentration of alkaloids. Correspondingly, the leaves displayed higher antioxidant properties (specifically, combatting lipid peroxidation) and a greater prevention of albumin denaturation than the stems. Both extracts inhibited hemolysis. Our findings provided valuable insight into the efficacy and applicability of plant compounds in the food and pharmaceutical industries.

Keywords: *Teucrium polium*; phytochemicals; antioxidant; hemolysis; anti-inflammatory
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INTRODUCTION

There is increasing awareness that the consumption of medicinal plants is critical for improving or maintaining health, considering their vast array of nutritional components that have been shown to influence the biochemical activities in the human body (Gill *et al*., 2011; Sofowora *et al*., 2013; Kathirvel and Sujatha, 2016; Veiga *et al*., 2020). Phytochemicals, including phenolics, flavonoids, alkaloids, saponins, terpenoids and carotenoids, noteworthily, exhibit antimalarial, antitumor, antimicrobial, antidiabetic and antiulcerogenic properties (Nunes *et al*., 2012). Moreover, antioxidants detected in plants (Ko *et al*., 2015; Pavithra *et al.*, 2016; Lourenço *et al*., 2019) have been shown to counter carcinogens (Rohman *et al*., 2010) and free radical scavengers in cellular systems (Kurutas, 2015). These substances trigger inflammation by generating reactive oxidative species, which often lead to tissue damage (Bishop, 2008; Aitken *et al.*, 2009) and the development of chronic diseases, such as cancer and rheumatoid arthritis (Sugimoto *et al*., 2016). Emerging evidence has revealed that plant compounds may be used as a promising preventive measure or for the treatment of free radicals (Karawya *et al*., 2010; Boubekri *et al*., 2014; Sagnia *et al*., 2014; Jamshidi-Kia *et al*., 2020) as they inhibit inflammatory molecules such as prostaglandin E_2, COX, 5-LOX, nitric oxide (NO), reduction in C-reactive protein (CRP), NFκB and various cytokines (Bajpai *et al*., 2014; Sagnia *et al*., 2014; Azab *et al*., 2016; Oguntibeju, 2018).

Teucrium (T) polium L. (Lamiaceae) is a wild flowering plant grown throughout Southwestern Asia, Europe and North Africa (Marzouk *et al*., 2016); it has been extensively utilized in traditional medicine for treating a wide range of pathological conditions (Stankovic *et al*., 2011; Jaradat, 2015; Hashemi *et al.*, 2020). Previous laboratory phytochemical screenings of this plant have identified bioactive compounds such as glycosides (verbascoside and poliumoside);

phenylethanoid; apigenin, 3',6-dimethoxy apigenin, 4',7-dimethoxy apigenin, rutin, flavonoids, tannins, terpenes and phenols (Sharififar *et al*., 2009; Bahramikia and Yazdanparast, 2012). These bioactive compounds exert cytotoxic, anticancer, antimutagenic, antioxidant and antibacterial effects on various cell lines (Capasso *et al*., 1984; Jurišić *et al*., 2003; Rajabalian, 2008; Khader *et al*., 2010; Shtukmaster *et al.*, 2010; De Marino *et al.*, 2012; El Atki *et al.*, 2020). Of particular, longstanding interests are their ability to safeguard against the rupturing of red blood cells and hepatocytes by decreasing the amount of hydrogen peroxide- and Fe^{2+}-induced lipid peroxidation, respectively (Suboh *et al*., 2004; Ljubuncic *et al*., 2005).

Currently, limited research has been conducted on *T. polium L* grown in Libya (Elmestiri, 2007; Abouzeed *et al*., 2013) and to our knowledge; no one has examined its specific antioxidant and anti-inflammatory profiles. Therefore, the purpose of this study was to identify bioactive compounds and assess the biological properties of samples grown in that country.

MATERIALS AND METHODS

Plant materials and alcoholic extraction: Fresh leaves and stems of *T. polium* were collected in August 2019 from Tarhwona, Libya. The collected plant was identified and authenticated by the Botany Department, Faculty of Science, University of Tripoli, Tripoli, Libya. The aerial parts (leaves and stems) of the plant were washed with tap water and air-dried at room temperature. The samples were ground into a powder, passed through a suitable mesh sieve and dried. The powdered plant parts were extracted with 95% methanol at 25°C for 48 h and after filtration, the samples were concentrated using a rotary evaporator (Heidolph, LaboRota 4000, Germany) under reduced pressure at 40°C. The residues were maintained at 20°C until analysis.

Chemicals: The chemicals, including 1,1'-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu phenol reagent, catechin, butylated hydroxyl anisole (BHA), coumarin, cortisone, phosphate buffered saline (PBS) and Anthron reagent were obtained from Sigma Chemical Company Ltd. (USA). Gallic acid, tannic acid, ascorbic acid, rutin, tannic acid, vitamin E (α-tocopherol), β-carotene and bovine serum albumin (BSA) were obtained from Merck (Pvt.) Ltd. (Germany). Solvents and other reagents were of analytical grade.

Quantifying ash content: Ash content was determined according to Horwitz and Latimer (2007) and expressed as %w.

Quantifying total protein content: Total protein content was estimated according to the method described by Lowry *et al*. (1951). The amount of protein in 100 mg of extract was calculated by comparison with the standard curve for BSA.

Quantifying total carbohydrate content: Total carbohydrate content was estimated using Anthron reagent, as described by Hedge *et al*. (1962).

Extracting free fatty acids and quantifying total lipid content: Free fatty acids were first extracted according to the method described by Bligh and Dyer (1959). The free fatty acids in the lipid residue were calorimetrically estimated using a cupric acetate/pyridine reagent, as described by Lowry and Tinsley (1976).

Quantifying total ascorbic acid (vitamin C) content: Total ascorbic acid content was estimated according to the technique described by Ghate *et al.* (2013) and calculated on the basis of the calibration curve of L-ascorbic acid and expressed as mg of ascorbic acid equivalent per g of extract.

Quantifying total tocopherol (vitamin E) content: Total tocopherol content were determined using the method described by Wong *et al*. (1988), calculations were based on a standard curve of α-tocopherol (10–100 mg/mL in toluene) and it was expressed as mg of α-tocopherol equivalent per g of extract (mg α-tocopherol E/g).

Quantifying total carotenoid content: Total carotenoid content of crude extracts was determined according to Gentili and Caretti (2011) and expressed as mg of β-carotene equivalent per g of extract (mg β-carotene E/g).

Quantifying total phenolic content: Total phenolic content was analysed using the Folin–Ciocalteu colorimetric method by Singleton *et al*. (1999) and expressed as mg of gallic acid equivalent per g of extract (mg GAE/g).

Quantifying total flavonoid content. Total flavonoid content was estimated according to the method described by Zhishen *et al*. (1999) and expressed as mg of rutin (Sigma Chemical Company Ltd., USA) equivalent per g of extract (mg RE/g).

Quantifying total flavonol content: Total flavonol content was determined according to the procedure by Kumaran and Karunakaran (2007) and expressed as mg of rutin equivalent per g of dry weight (mg RE/g).

Quantifying total tannin content: Total tannin content was determined according to the method detailed by Julkunen-Tiitto (1985) and expressed as mg of tannic acid equivalent per g of dry weight (mg TAE/g).

Quantifying total alkaloid content: Total alkaloid content was determined according to the method described by Shamsa *et al*. (2008) and Sharief *et al*. (2014) and expressed as mg of atropine equivalent per g of extract (mg AE/g).

Quantifying total coumarin content: Total coumarin content was estimated following the standard methods by Rajat Buragohain (2015) and de carvalho Osório and Martins (2004) and expressed as mg of coumarin equivalent per g of extract (mg CE/g).

Quantifying total steroid content: Total steroid content was estimated according to Devanaboyina *et al*. (2013) and expressed as mg of cortisone equivalent per g of extract (mg QE/g).

Assessing *in vitro* biological activities

Assessing antioxidant actions: Investigations of *in vitro* antioxidant activity were according to different assays that examined their behaviour as radical scavengers or reducing agents.

Total antioxidant capacity was determined according to the procedure proposed by Prieto *et al*. (1999) at various concentrations (6.25–100 mg/mL).

The reducing power was determined according to the method detailed by Oyaizu (1986)) at various concentrations (6.25–100 mg/mL).

The free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazine (DPPH) was evaluated as described by Wong *et al*. (2006).

The nitric oxide radical was measured spectrophotometrically according to Garratt (2012) at various concentrations (6.25–100 mg/mL).

Lipid peroxidation was evaluated according to Kuda *et al*. (2005), at various concentrations (6.25–100 mg/mL). The scavenging activity of the DPPH and nitric oxide radicals as well as the inhibition of lipid peroxidation were calculated as percentages using the following equation (where A_C was the absorbance of the control reaction and A_S was the absorbance in the presence of the extracts)

$$(\%) = [(A_C - A_S)/A_C] \times 100$$

Note: In all assays, ascorbic acid and butylated hydroxyl anisole (BHA) at various concentrations (6.25–100 mg/mL) were used as positive controls. The IC_{50} was calculated as the number of antioxidants required to inhibit 50% of the radical.

Assessing anti-inflammatory actions

Inhibiting albumin denaturation: This assay was performed according to the method detailed by Williams *et al*. (2008) at various concentrations (6.25–100 mg/ml) based on inhibition of albumin denaturation. Ascorbic acid and (BHA) were used as positive controls. The inhibition percentage of protein denaturation was calculated as follows (where D was the absorbance reading of the test sample and C was the absorbance reading without the test sample):

% Denaturation inhibition = (1 - D/C) ×100%.

Evaluating *in vitro* antihemolytic activity: Antihemolytic activity was assessed using human erythrocytes (O blood groups) as suggested by Takeshima *et al*. (2003) at various concentrations (6.25–100 mg/mL). A saline buffer was used as the negative control, and Triton X-100 was used as the positive control. Hemolysis percentage was determined as follows:

% Hemolysis = $[(A_{\text{sample}} - A_{\text{buffer saline}})/ (A_{\text{Triton X-100}} - A_{\text{buffer saline}})] \times 100$.

Statistical analyses: All the assays were repeated three times (n = 3), and values were expressed as mean ±Margin of Error (MOE). Statistical analysis was performed using SPSS (Statistical Program for Social Sciences) v.16 (SPSS Corporation, Chicago, IL). *P* values of ≤0.05 were considered statistically significant. Statistical significance was assessed using one-way ANOVA for the average over the time, followed by Tukey's multiple comparisons test with a significance level set at $p \leq 0.05$. Pearson correlation coefficient was determined between the antioxidant activities and primary, secondary metabolite contents.

RESULTS AND DISCUSSION

***In vitro* antioxidant activity:** The antioxidant capacity of methanolic extract of leaves and stems was evaluated using several different methods based on two mechanisms, reducing capacity and free radical scavenging, to enable quick screening of compounds as tested samples that possess a reduced antioxidant impact *in vitro* will possibly show less effect *in vivo* (Nunes *et al*., 2012).

The phytochemical and biological properties of methanolic extracts of leaves and stems of *T. polium* grown in Libya were investigated. Specifically, we qualitatively and quantitatively evaluated their nutritive and non-nutritive (i.e. phytochemical) compounds as well as overall antioxidant, antihemolytic and anti-inflammatory activities.

Nutrients and phytochemical analyses: Phytochemical screening revealed the presence of different bioactive compounds and nutrients (Table 1). Phenols, tannins, glycosides, flavonoids, alkaloids, coumarins, terpenoids, steroids, resins, anthraquinones, emodins, proteins, carbohydrates and fats were found to be present in both leaf and stem extracts. The most abundant bioactive compounds were steroids, coumarins, terpenoids and resins (+++), followed by polyphenols, tannins, glycosides, flavonoids and fats (++), and then flavonols, proanthocyanidins, anthraquinones, emodins, glycosides, carbohydrates and proteins (+). Alkaloids and quinones were present in appreciable amount in the leaf extract (++) compared with that in the stem extract (+). Catechins were present only in the leaf extract (+). These

findings are consistent with related previous phytochemical studies reporting the presence of tannins, diterpenoids (Piozzi *et al*., 2005), flavonoids (D'Abrosca *et al*., 2013), iridoids and teucardoside (Elmasri *et al*., 2015). Interestingly, our samples showed that saponins were absent, which is inconsistent with the results of (Elmasri *et al*., 2016), indicating that the composition of the samples is influenced by their geographic origin.

Table 1. Qualitative analysis of phytochemicals in leaves and stems of *Teucrium polium.*

Phytochemicals	Methanol extract	
	Leaf	Stem
Phagzenols	++	++
Tannins	++	++
Phlobatannins	−	−
Flavonoids	++	++
Flavonols	+	+
Anthocyanin	−	−
Anthocyanidins	−	−
Proanthocyanidins	+	+
Catechins	+	−
Coumarins	+++	+++
Alkaloids	++	+
Saponins	−	−
Terpenoids	+++	+++
Steroids	+++	+++
Quinones	++	+
Anthraquinones & Emodins	+	+
Resins	+++	+++
Glycosides	+	+
Carbohydrates	+	+
Proteins	+	+
Fats/ Fixed oils	++	++

+++ Copiously present, ++ Moderately present, + Slightly present, - Absent

Nutrient contents: Apart from the potential use of medicinal plants as pharmacological agents, they can also serve as a source of food in countries wherein malnutrition is prevalent (Adesogan *et al*., 2020) owing to their rich nutrition. The nutrient contents of the leaves and stems tested in our study are summarised in Table 2. Ash content was greater in the leaves than in the stems ($P \leq 0.05$). In general, the leaf was the major source of nutrition, and the amounts of these components, from greatest to least, are as follows: Vitamin C > carbohydrates > proteins > vitamin E > fats > carotenoids. These findings are inconsistent with those of previous research and it could be owing to differences in geographical regions, condition and structure of soil, environment, features, genetics, different parts of the plant and period of assessment (Imeh and Khokhar, 2002; Maqsood *et al*., 2020).

Phytochemical contents: The concentration of the various phytochemicals in the leaves and stems of *Teucrium polium* is presented in Table 3. Phenolic content. Phenols are widely found in medicinal plants and are proven to have strong antioxidative properties (Santos-Sánchez *et al*., 2019). In our study, the leaves had a significantly higher concentration of phenols than the stems ($P \leq 0.001$). In previous studies, the phenolic content in leaves was found to be less than that found in this study (Stankovic *et al*., 2011; Stankovic *et al*., 2012).

Tannin content: Tannins exhibit many antioxidant, antimicrobial and anti-inflammatory properties (de Sousa Araújo *et al*., 2008; Amabeoku, 2009; Corrales *et al*., 2009; Hashemi *et al*., 2020). In our study, the concentration of tannins in leaves was significantly higher than that in stems (0.001). Notably, the content of tannins was the lowest compared with that of other identified phytochemical compounds (Table 3).

Flavonoid and flavonol contents: Extensive research has demonstrated a good correlation between flavonoid amounts and the degree of bioactivity in plants (Cakir *et al*., 2003; Al-Shalabi *et al*., 2020). In our study, the flavonoid content of the leaves was significantly higher than that of the stems ($P \leq 0.008$). These findings are consistent with the results obtained by Bendjabeur *et al*. (2018), although the content of flavonoids in their leaves was almost twice as high as the amount detected in this study. Similarly, regarding the content of flavonols, it was markedly higher in the leaves than in the stems ($P \leq 0.0001$) (Table 3).

Table 2. Nutritional contents (%) of leaves and stems of *Teucrium polium.*

S. No	Parameters (%)	Plant Part		*P* value
		Leaf	Stem	
1	**Ash**	16.62 ± 0.32	10.37 ± 0.22	0.05
2	**Moisture**	ND	ND	-
3	**Carbohydrates**	6.91 ± 0.53^{a}	6.41 ± 0.50^{a}	0.183
4	**Proteins**	1.58 ± 0.17^{b}	0.25 ± 0.10^{a}	0.0001
5	**Fats**	0.12 ± 0.02	0.05 ± 0.01	0.0001
6	**Vitamin C**	25.53 ± 0.62^{a}	20.36 ± 1.83^{b}	0.006
7	**Vitamin E**	0.26 ± 0.11^{a}	0.20 ± 0.10^{a}	0.468
8	**Carotenoids**	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}	0.124

Data are expressed as means ± MOE (n=3). Values with different superscripts are significantly different.

Table 3. Phytochemical content of leaves and stems of *Teucrium polium.*

S. No	Phytochemical	Unit	Plant Part		*P* value
			Leaf	Stem	
1	**Phenols**	mg GAE/g	2579.72 ± 81.31	1632.57 ± 77.19	0.0001*
2	**Tannins**	mg TAE/g	1.26 ± 0.05	0.58 ± 0.12	0.001*
3	**Flavonoids**	mg RE/g	52.63 ± 4.79	25.97 ± 0.33	0.008*
4	**Flavonols**	mg RE/g	4.32 ± 0.28	1.72 ± 0.35	0.0001*
5	**Catechins**	mg CaE/g	1.36 ± 0.28	-	-
6	**Coumarins**	mg CoE/g	45.68 ± 3.95	26.49 ± 4.48	0.006*
7	**Alkaloids**	mg AE/g	0.52 ± 0.26	1.46 ± 0.23	0.003*
8	**Steroids**	mg QE/g	107.84 ± 2.58	105.21 ± 3.01	0.236

Data are expressed as means ± MOE (n=3). Gallic acid, TA = Tannic acid, R = Rutin, Ca = Catechin; Co= coumarins A= atropine; Q: cortisone *significantly $P \leq 0.0$.

Catechin content: Catechins have proven to be effective in preventing lipid oxidation, and therefore may be particularly useful in pharmaceutical products and extending the shelf life in food products (Wong *et al*., 2006). In our study, catechins were detected only in the leaves. In a related study, catechins were detected in the aerial parts of the plant. However, the amounts were lower than found in our study (Saif-Elnasr *et al*., 2019), presumably owing to estimations using HPLC (Table 3).

Coumarin content: Coumarins have been used to treat diseases owing to their low levels of toxins and side effects (Wang *et al*., 2009) and their anticoagulant, antioxidant, antimicrobial, anticancer, antidiabetic, analgesic and anti-inflammatory properties (Bansal *et al*., 2013; Borges Bubols *et al*., 2013; Matos *et al.*, 2013; Xia *et al*., 2013). In this study, the content of coumarins in the leaves was significantly higher than that of the stems ($P \leq 0.006$) (Table 3). This finding was lower than that reported by Purnavab *et al*. (2015).

Alkaloid content: Alkaloids possess antioxidant, antibacterial, anti-plasmodial, anticancer and anti-inflammatory properties (Alghazeer *et al*., 2013; Thawabteh *et al*., 2019). In our results, the level of alkaloids was significantly higher in the stems than in the leaves ($P \leq 0.001$) (Table 3).

Steroid content: Steroid compounds have been isolated from natural sources and their bioactivities have been investigated (Ulubelen *et al*., 2000). The amount of steroids in leaves was greater than that in stems (Table 3).

Reducing power and total antioxidant activity: Examining the reducing power and total antioxidant activity are simple techniques that are used for preliminary estimation of a sample's ability to combat free radicals. Previous studies have demonstrated a positive relationship between phenolic and flavonoid contents and antioxidant activity in many plants (Oktay *et al*., 2003; Sharififar *et al*., 2009). Dose response curves of the reducing powers and antioxidant activity of the leaves and stems of *Teucrium polium* are presented in Figure 1a & b. It is noteworthy that the reducing power and total antioxidant activity were dependent on the concentration of extracts, wherein the highest effects were observed at the highest concentration (100 mg/mL). Interestingly, the reducing power for our extracts was lower than that observed for positive controls (Ascorbic acid and BHA). No significant differences were observed between our present findings and those of a study by (Stankovic *et al*., 2012).

Free radical scavenging and anti-lipid peroxidation activities: Free radical scavenging activity using DPPH and NO radicals are presented in Figure 2a & b. Our results showed that the percentage of radical scavenging activity increased with increasing concentrations of the extracts. Maximum DPPH radical scavenging ability observed in the leaves and 28% in the stems. Across all concentrations, the leaves showed significantly higher DPPH scavenging activity than the stems ($P \leq 0.05$). Specifically, the IC_{50} values for the DPPH scavenging activities, from greatest to least, are as follows: Asc > BHA > leaves > stems. Interestingly, the DPPH scavenging activity of the extracts was significantly lower than those of BHA and ascorbic acid ($P \leq 0.05$) (Table 4). The same pattern of results was obtained using NO radicals, although our extracts showed a stronger effect against NO radicals than DPPH radicals. Both extracts showed minimal radical scavenging activity compared with positive controls. These findings are in line with those of previous studies (De Marino *et al*., 2012; Elmasri *et al*., 2017).

The anti-lipid peroxidation activity is shown in Figure 2c. The obtained results are similar to those of the DPPH and NO radicals scavenging activities, wherein the inhibition percentage of lipid peroxidation was dependent on the concentration of the extracts, and the leaves were better at inhibiting lipid peroxidation than the stems. Maximum inhibition of lipid peroxidation was exhibited by the leaves (Table 4). These results are consistent with those of related investigations (Panovska and

Kulevanova, 2005; Krishnaiah *et al*., 2011; Vladimir-Knežević *et al.*, 2014).

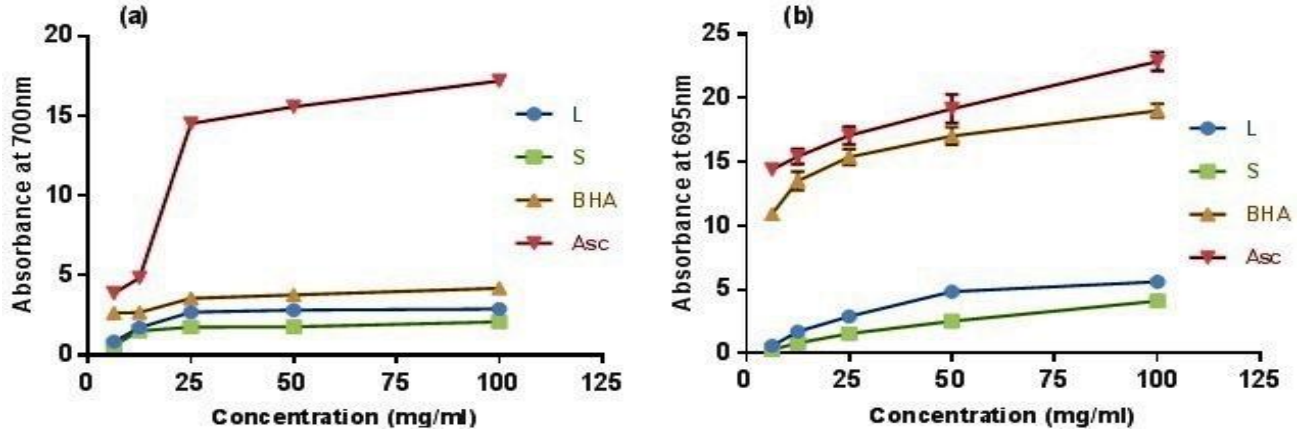

Fig. 1. Reducing power and total antioxidant activity of leaves and stems of *Teucrium polium*. Data are expressed as mean ± MOE (n = 3): (A) Reducing power, (B) Total antioxidant activity. L: leaves, S: stems, BHA: Butylated hydroxyanisole, Asc: Ascorbic acid.

The NO and DPPH free radical scavenging activities as well as the anti-lipid peroxidation activity of the samples are also expressed as the inhibitory concentration IC_{50} in Table 4. IC_{50} values for the DPPH scavenging activities, from greatest to least, are as follows: Asc > BHA > leaves > stems. A similar pattern was observed for the NO scavenging and anti-lipid peroxidation activities. In comparison with ascorbic acid or BHA, the activity of the extracts was significantly lower ($P \leq 0.05$). According to (Phongpaichit *et al*., 2007), extracts with IC_{50} values ranging between 10 and 50 mg/mL were considered to possess strong antioxidant activity, thereby confirming that our stems and leaves are powerful scavengers of free radicals.

Table 4. IC_{50} values for NO and DPPH free radical scavenging activities as well as the anti-lipid peroxidation activity of leaves and stems of *Teucrium polium.*

Samples	IC_{50} (mg/mL)		
	NO˙	DPPH˙	Anti-LP
Leaf	9.375^{d}	6.25^{c}	12.5^{b}
Stem	12.5^{c}	12.5^{d}	15.625^{c}
BHA	4.5^{b}	3.125^{b}	12.5^{b}
Asc	0.15625^{a}	0.78125^{a}	6.25^{a}

Data are expressed as actual mean (n = 3): (A) DPPH (1,1-diphenyl-2-picrylhydrazine) radical scavenging activity, (B) NO (nitric oxide) radical scavenging activity, (C) lipid peroxidation. L: leaves, S: stems, BHA: Butylated hydroxy anisole, Asc: Ascorbic acid. Values with different superscripts are significantly different.

Antihemolytic activities: Erythrocyte membranes, due to the presence of polyunsaturated fatty acids, are the main target of free radicals (Fibach E, 2014). In an investigation examining hemolytic anaemia, researchers found that oxidative stress led to the induction of hemolysis (Fibach and Rachmilewitz, 2008). However, numerous studies have shown that plant compounds such as polyphenols and flavonoids are capable of safeguarding against such stress (Asgary *et al.*, 2005; Kalaivani *et al*., 2011; Naqinezhad *et al.*, 2012). Table 5 shows that the hemolysis induced by the leaves and stems in our study on red blood cells occurred in a concentration-dependent manner. Overall, the leaves showed a lower hemolytic effect than the stems, although the difference was not significant. In comparison with Triton X-100 (known to cause impressive red blood cell by membrane swelling and subsequent hemolysis), the extracts here displayed significantly lower hemolysis, indicating a low toxicity (Amrani *et al*., 2006). These results are consistent with those of (Suboh *et al.*, 2004).

Inhibition of albumin denaturation: It has been reported that protein denaturation is associated with inflammatory diseases such as arthritis; therefore, identifying substances that inhibit this denaturation are of great interest (Reddy *et al*., 2014). Figure 3 shows that the leaves and stems are both effective at inhibiting heat-induced albumin denaturation in a concentration-dependent manner. Of interest, the leaves were more potent than the stems ($P \leq 0.05$), and no significant differences were observed with regard to the positive controls. These findings are consistent with previous observations (Mehrabani *et al*., 2009; Shah *et al*., 2012).

Correlations among nutrients, phytochemicals and the biological activities: Table 6 shows correlations among nutrients, phytochemicals and the biological activities. For the leaves, DPPH˙ activity was positively correlated with phenols and coumarins ($P \leq 0.05$). A positive correlation was found between NO˙ activity and

fat content ($P \leq 0.024$). The reducing power in the leaves was positively correlated with the concentrations of carbohydrates, proteins and flavonoids ($P \leq 0.05$). Anti-protein denaturation was positively correlated with tannins ($P \leq 0.986$). For the stems, DPPH activity was negatively correlated with carotenes ($P \leq 0.02$) and fat content ($P \leq 0.007$). The total antioxidant capacity (TAC) was negatively correlated with tannins ($P \leq 0.05$) and coumarins ($P \leq 0.006$). The present findings are in agreement with those of previous studies (Felhi *et al*., 2016; Gan *et al*., 2017; Sayyad and Farahmandfar, 2017; Petropoulos *et al*., 2018). Bioactivity assays indicate that nutrients and phytochemicals exhibit redox properties, which allow them to act as reducing agents.

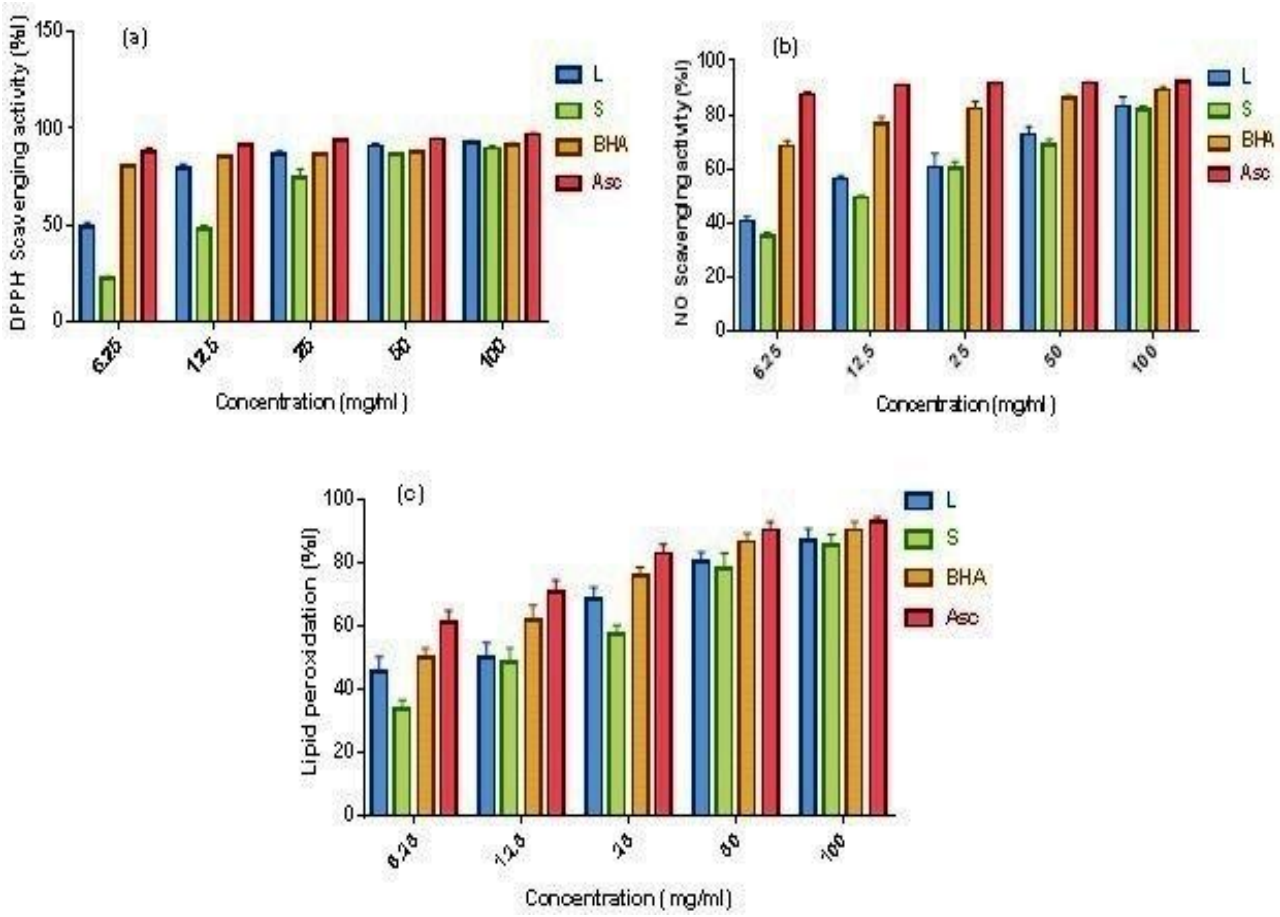

Fig. 2. Free radical scavenging and anti-lipid peroxidation activities of leaves and stems of *Teucrium polium*. Data are expressed as mean ± MOE (n = 3): (A) DPPH (1,1-diphenyl-2-picrylhydrazine) radical scavenging activity, (B) NO (nitric oxide) radical scavenging activity, (C) Lipid peroxidation activity. L: leaves, S: stems, BHA: Butylated hydroxyanisole, Asc: Ascorbic acid.

Table 5. Antihemolytic activity of leaves and stems of *Teucrium polium*..

Concentration (mg/ml)	Hemolysis activity (%)	
	Leaf	Stem
6.25	1.67 ± 0.03^{a}	2.13 ± 0.08^{a}
12.5	2.00 ± 0.09^{a}	2.40 ± 0.09^{a}
25	2.33 ± 0.76^{a}	3.13 ± 0.09^{a}
50	3.01 ± 0.15^{a}	4.27 ± 0.12^{a}
100	4.86 ± 0.52^{a}	5.13 ± 0.19^{a}
0.1% Triton X-100	99.33 ± 1.30^{c}	
8.5 % Buffer saline	0.04 ± 0.03^{b}	

Data are expressed as means ± MOE (n=3). Values with different superscripts are significantly different ($P \leq 0.01$)

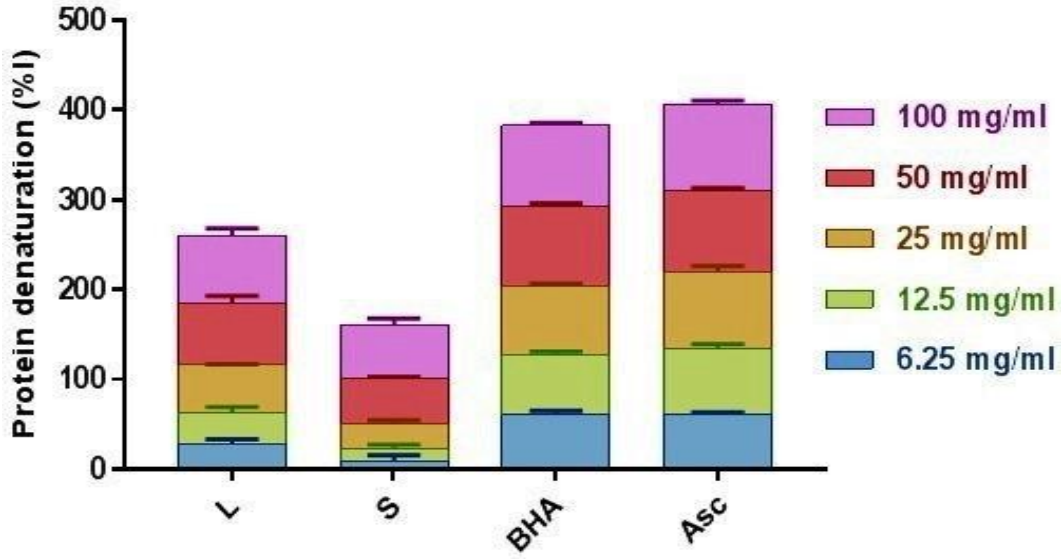

Fig. 3. Inhibition of albumin denaturation by leaves and stems of *Teucrium polium*. Data are expressed as means ± MOE (n=3). L: leaves; S: stems; BHA: Butylated hydroxyanisole; Asc: Ascorbic acid.

Table 6. Correlations between nutrients, phytochemicals and the biological activities in leaves and stems of *Teucrium polium*.

	DPPH˙	NO˙	Anti-PD	Anti-LP	TAC	RP
			Leaf Extract			
Carbohydrates	0.59	0.133	−0.978	−0.947	0.054	0.997*
Proteins	0.623	0.092	−0.968	−0.933	0.095	0.999*
Fats	−0.695	0.999*	−0.374	−0.478	−0.975	0.094
Vitamin C	−0.954	0.896	0.115	0.000	−0.963	−0.392
Vitamin E	−0.989	0.816	0.268	0.156	−0.910	−0.531
Carotenes	−0.760	0.098	0.904	0.849	−0.281	−0.988
Polyphenols	0.999*	−0.686	−0.453	−0.348	0.809	0.688
Tannins	−0.378	−0.368	0.999*	0.997	0.189	−0.949
Flavonoids	0.667	0.034	−0.952	−0.911	0.152	1.000*
Flavonols	0.326	−0.889	0.731	0.804	0.789	−0.506
Catechins	−0.851	0.251	0.826	0.756	−0.427	−0.952
Alkaloids	−0.553	−0.178	0.986	0.961	−0.009	−0.993
Coumarins	0.997*	−0.667	−0.476	−0.372	0.794	0.707
			Stem Extract			
Carbohydrates	−0.755	−0.312	0.264	0.921	0.934	−0.792
Proteins	−0.562	0.98	0.926	0.277	−0.466	−0.512
Fats	−1.000**	0.378	0.826	0.954	0.479	−0.999*
Vitamin C	−0.248	0.989	0.744	−0.065	−0.739	−0.190
Vitamin E	0.668	0.427	−0.143	−0.866	−0.971	0.711
Carotenes	−0.999*	0.417	0.85	0.94	0.441	−0.996
Polyphenols	0.586	−0.974	−0.937	−0.305	0.44	0.537
Tannins	0.536	0.571	0.023	−0.771	−0.997*	0.585
Flavonoids	0.668	0.427	−0.143	−0.866	−0.971	0.711
Flavonols	−0.289	0.994	0.772	−0.023	−0.710	−0.232
Alkaloids	0.034	−0.935	−0.583	0.277	0.866	−0.024
Coumarin	0.462	0.639	0.108	−0.714	−1.000**	0.513

DPPH˙: 1,1-diphenyl-2-picrylhydrazine radical activity, NO˙: nitric oxide radical activity, Anti-PD: anti-protein denaturation, Anti-LP: anti-lipid peroxidation, TAC: total antioxidant activity, RP: reducing power

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed)

Conclusion: The nutritive and phytochemical compositions of *Teucrium polium* leaves extract are quite remarkable in contrast to their content in stems extract. Leaves extracts showed considered amount of carbohydrates, vitamin C, polyphenols, flavonoids, flavonols, and coumarins. In addition, the *in vitro* antioxidant efficacy of the extracts showed a dose-dependent effect, since decreasing concentrations of the extract had decreasing reducing and scavenging free radicals' abilities as well as decreasing hemolysis and protein denaturation activities. Moreover, the results showed that noticeable correlations between the concentration of some nutrients, phytochemicals and the biological activities. Our findings provide valuable insight into the efficacy and applicability of *Teucrium polium* compounds in the food and pharmaceutical industries.

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