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Anti-inflammatory and Antioxidant Activities of *Teucrium polium* Leaf Extract and its Phenolic and Flavonoids Content

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Teucrium polium leaf is one of the folkloric medicinal plants used traditionally to treat many diseases in the Palestinian community. It has antibacterial antioxidant and anti-inflammatory consequences. Present study aims the evaluating the *in vitro* inhibitory effect of *Teucrium polium* leaf extracts on tumor necrosis factor-α (TNF-α) using polymorphonuclear cells (PMNCs), in addition to determine its antioxidant and total phenolic and flavonoids contents. Polymorphonuclear cells were withdrawn from whole blood according to Histopaque (Ficol-1077) method. Blood cells were cultured in an enriched Roswell Park Memorial Institute (RBMI) medium. The levels of tumor necrosis factor (TNF-α) were determined 24 h using LPS stimulation. Total phenolic contents, flavonoids contents and antioxidant activity were measured using spectrophotometric method. The TNF-α concentrations were compared using paired-samples t test. The leaf extracts of *Teucrium polium* revealed significant reduction in terms of TNF-α levels. The extract contained high phenolic and flavonoids contents and its antioxidant activities were strong. The reduced values in the TNF-α levels as affected by *Teucrium polium* leaf extracts indicate its effect in anti-inflammation. The plant is rich with polyphenolic compounds and flavonoids and has strong antioxidant activity. The observed anti-inflammatory effect of the extracts under study may be discussed as the influence of the significant presence of the phenolic compounds and flavonoids.

Keywords: *Teucrium polium*, Plant extracts, TNF-α, Anti-inflammatory effect.

INTRODUCTION

Folkloric medicine had been used for a long time in all around the world. Palestine is among the areas that are famous in using the herbs to treat many diseases. *Teucrium polium* (TP) is a well-known native Palestinian plant. It belongs to *Lamiaceae* family and has many species that is thought to recur many diseases such as diabetes and some liver disorders. It is used to alleviate pain related with coughing, miscarriage and pregnancy [1-3]. *T. polium* was among many medicinal plants that have been used to treat rheumatism, inflammations, indigestion and common cold.

Many different compounds were isolated from the medicinal plant under investigation including flavonoids and terepenoids. Such compounds are well known in their pharmacological effects such as hypoglycemic, anti-inflammatory, heptoprotective, antifungal, antibacterial and hypolipidimic [4].

Different components of *Teucrium polium* have indicated anticancer activities against many types of tumors. Such effect was shown in some studies on different types of cancer cells as MDA-MB-231 and MCF-7 breast carcinoma, epidermoid carcinoma (A431), Saos-2 osteoblastoma, K562 chronic myelogenous leukemia, SW480 colon carcinoma, BT20 human breast ductal carcinoma, K562 chronic myelogenous leukemia, A549 human lung adenocarsinoma cell lines and PC12 mouse pheochromocytoma and REYF-1 glioblastoma multiforme [5-8].

Previous studies documented the effect of the studied plant extract on the male reproductive system. The aqueous extract of *T. poliuom* has increased the testosterone levels, testicular weight, spermatogonia, spermatozoa and leyding cells in the

treated groups [8]. In the other hand, chronic treatment with the *T. polium* ethanolic extract led to a clear reduction in the mice testes' weight as well as increase in sperm abnormalities. Also, glucose levels were decreased compared to the control treatment [9]. The essential oil of the *T. polium* has shown an antibacterial activity against resistant microorganisms as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* [10,11].

Although high number of herbs and parts of trees used worldwide in folkloric therapy, few of them were tested pharmacologically and phytochemically for pharmaceutical applications. Plenty of the active ingredients reported out of medicinal plants may carry out antimicrobial, anti-inflammatory and free radicals scavenging action. Biologically active ingredients may include phenolic compounds, anthocyanins, caratenoids and thiols [12,13].

Inflammation response is part of the innate immunity used by human body against invading pathogens. Therefore helps in healing injured tissues. Pro-inflammatory cytokines (interlukin-6 (IL-6) and tumor necrosis factor (TNF-α)) may create injury to normal tissues at the time of inflammation process within the human tissues. Excessive production of these cytokines may emerge into chronic inflammatory diseases as asthma, rheumatoid arthritis and atherosclerosis. Drugs having anti-inflammatory activity decrease such proinflammtory cytokines production and therefore enhance the symptoms of inflammation [14-15].

Tumor necrosis α and interlokin-6 are produced by the monocytes, T-cells, B-cells, endothelial cells and other cells as pro-inflammatory mediators. The release of these pro-inflammatory cytokines could be stimulated by lipopolysacharide (LPS) of Gram-negative bacteria as an endotoxin and part of an outer cell membrane component of these bacteria. Therefore, LPS triggers inflammation and may cause septic shock [16-18]. The anti-inflammatory effect of *T. polium* has not been extensively investigated. In present research, we have focused on anti-inflamatory, antioxidant activities in addition to the determination of the contents of total phenolic compounds and total flavonoids of *T. polium* leaves extracts.

EXPERIMENTAL

Plant material and extraction: *Tecurium polium* plant was collected in April 2020. The plants were air-dried in shade for 2 weeks, then were grinded. Grinded material (50 g) was mixed with 500 mL of 96% ethanol and left on the shaker for 5 days. The mixture was filtered through Whatman filter paper. Using rotary evaporator at 50 ºC, the filtrate was dried leaving the extract.

Isolation of whole blood polymorphonuclear cells: Whole blood from an adults healthy person was transfused, from which, a 5 mL was freshly collected in an EDTA tube and then diluted with equal volume of phosphate buffered saline (PBS) under completely sterile condition. The diluted blood was gently mixed. Consequently, 3 mL Histopaque (Ficol-1077) were pipetted into a sterile, 15 mL conical tube. The blood and PBS mixture were added gently to the Histopaque and the tube was spun for 20 min at 400 g. The mixture was separated into four distinct layers: red blood cells, Ficol layer, polymorphonuclear cells (PMNCs) and PBS and the plasma from lower to upper layer.

The polymorphonuclear cells were aspirated and washed with 10 mL of PBS in 12 mL conical tubes for three times at 100 g for 10 min each time. The supernatant was discarded and the PMN cells were collected.

Cell culture: The poly morpho nuclear cells were isolated and treated to investigate the anti-inflamatory effect of the extract according to Qabaha *et al*. [12].

Cytotoxicity test: Toxicity of the *T. polium* extract was evaluated using the trypan blue exclusion test according to Avelar-Freitas *et al.* [19].

Determination of total phenolic content: A reaction mixture of 0.2 mL of extract (5 mg/mL), 1 mL of diluted Folin-Ciocalteu's reagent and 0.8 mL $NaHCO_3$ (7.5%) was incubated at 45 ºC for 45 min. Gallic acid (GA) was used as a standard and total phenolic contents were expressed in terms of gallic acid equivalents (mg of GA/g of extract).

Determination of total flavonoids content: To 1 mL of extract, 4 mL of distilled water, 0.3 mL of 10% $AlCl_3$ and 0.3 mL of 5% $NaNO_2$ was added. After 6 min, 2 mL of 1 N NaOH and 2.5 mL of distilled water were added to the mixture, then was measured for absorbance at 510 nm. Results were expressed in mg catechin/g. Calibration curve of different concentrations of catechin was prepared and the absorption was measured at 510 nm.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method: The total antioxidant activity of the extract was assayed using DPPH as follows: Aliquots of various concentrations of the extract (0 to 2000 µg/mL) were added to 1 mL of 0.004% methanol solution of DPPH. Samples were incubated for 30 min at room temperature, then absorbance was measured at 517 nm. All determinations were done in triplicate. Inhibition of free radical scavenging activity was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_1 - \text{Abs}_2}{\text{Abs}_1} \times 100 \quad (141)$$

where, Abs_1 is the absorbance of the negative control which is a solution of 100 µL methanol 95% and Abs_2 is the absorbance of the positive control.

The concentration of the extract that give 50% inhibition (IC_{50}) was determined from a graph plotting percentage inhibition against extract concentration. Trolox was used as a standard, in the concentration range of 0-100 µg to construct a calibration curve and DPPH radical-scavenging activities were expressed as µg Trolox equivalents per mL of plant extract.

Ferric reducing antioxidant power (FRAP): This assay is a measure of the ability of the antioxidants to reduce ferric ions to the ferrous ions. To prepare a fresh FRAP reagent, 10 mM TPTZ (1 mL) and 20 mM ferric chloride (1 mL) in 0.25 M acetate buffer (10 mL, pH 3.6) were mixed together. The plant extract (50 µL) was added to 3 mL FRAP reagent obtaining a final concentration of 100 µg/mL. The absorbance of the samples (in triplicate) was measured after 8 min of incubation (room temperature) at 593 nm. This antioxidant capacity of the plant extract was calculated as µg Trolox equivalents per g of extract.

Statistical analysis: All statistical analyses were performed using SAS (SA Institute Inc., Cary, USA, Release 8.02, 2001). Means comparisons between different concentrations of TNF-α were tested using the GLM procedure. The Bonferoni test was employed with multiple t-test to maintain an experiment-wise of 5%. Results were shown as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Cytotoxicity of the extracts: Lipopolysaccharide (LPS) at concentration of 1 µg/mL and *Tecurium polium* extracts concentration of 500 µg/mL have no significant effect on the PMN cells viability as shown in Table-1.

TABLE-1
EFFECTS OF *T. polium* EXTRACTS AND LPS ON VIABILITY OF PMNCs

Treatment	Viability (%)
PMNCs only	96.3
PMNCs with LPS	94.5
PMNCs with LPS and 500 µg/mL of *T. polium* extract	91.0

Anti-inflammatory activity of plant extract: The measurement of the level of TNF-α by the mono nucleated white blood cells corresponding to the effect of LPS at different concentrations indicate the anti-inflammatory effect of the plant extract. Concentrations of the cytokines were evaluated using Enzyme Linked Immune Sorbent Assay (ELISA) method.

The level of the TNF-α produced by LPS stimulated PMNCs after 24 h has increased significantly. However, after treatment with 250, 500 and 1000 µg/mL extract of *T. polium* in the cell culture, the TNF-α levels were reduced significantly indicating the strong anti-inflammatory effect of this extract. Results are illustrated in Table-2.

TABLE-2
T. polium EXTRACT EFFECT ON PMNCs RELEASE OF TNF-α

Treatment	TNF-α value (pg/mL)	
	Average	STD
Cells only	111	1.4
Cells with LPS	591	1.4
Cells with LPS and 250 µg extract	40.5	1.7
Cells with LPS and 500 µg extract	10.5	0.8

Free radical scavenging activity of *T. polium* extract: To explore the antioxidant potential of the *T. polium*, the extract was analyzed for their capacity to scavenge oxidative radicals. The DPPH radical scavenging potential and FRAP of *T. polium* extract were assessed and compared to the positive control (Trolox) and expressed as TEAC (µg Trolox/g of plant extract). The TEAC for the extract was found to be 73.13 µg/g and IC_{50} was 15.1 µg/g for plant extract by using DPPH (Table-3). Similarly, with respect to FRAP radical scavenging activity, the plant extract had 6.41 TEAC (µg Trolox/g of plant extract).

Total phenolics and flavonoids content: The ethanolic extract yield, the total phenolic and flavonoids content of the plant extract is presented in Table-3. In this study, *T. polium* extract show higher concentration of phenol concentration with

TABLE-3
TOTAL FLAVONOIDS CONTENT (mg CA/g PLANT EXTRACT), TOTAL PHENOLIC COMPOUNDS (mg GAE/g PLANT EXTRACT)), DPPH SCAVENGING ACTIVITY (µg TEAC/g), FRAP ACTIVITY (µg TEAC/g) AND % YIELD OF *T. polium* ETHANOLIC EXTRACT

Yield* (%)	Total flavonoids	Total phenolic content	DPPH**	FRAP**
8.3	67.2 ± 1.5	155.2 ± 3.4	73.1 ± 5.2	6.41 ± 0.71

*Percentage extraction product (%) is represented as w/w g of dried extract. **DPPH radical scavenging activity and FRAP activity of extract is expressed as µg Trolox equivalent/g of plant extract.

155.2 mg GAE/g extract and high flavonoids content (67.2 mg CA/g).

Traditionally, *T. polium* medicinal plant has strong reputation for treating many diseases [1,2]. This work agrees with previous studies in which phytochemical analysis of *T. polium* showed the presence of alkaloids, flavonoids, terpenoids, tannins, such compounds have a vital medicinal role against various diseases [1-4]. This study demonstrated that *T. polium* is rich in phenolic compounds, which are considered very important components for their antioxidant activity, antibacterial, anticancer, antiviral and anti-inflammatory activities [20]. Antioxidants are molecules that suppress oxidation reactions by quenching free radicals and hence, protects the cell or delay its damage [20,21]. Natural antioxidant such as phenolic compounds (cinnamic acids, benzoic acids, flavonoids, coumarins, lignans and lignins), ascorbic acid and carotenoids are secondary metabolites produced in significant amounts by medicinal plants [21-23].

Many types of antioxidant tests are frequently used to evaluate antioxidant activity of medicinal plant extracts. Most of these methods depend on either measuring the potential of plant to reduce oxidant such as FRAP assay or to scavenge free radicals such as DPPH. The % of inhibition of DPPH at different concentrations of crude extract was found to be a dose dependent. The DPPH assay showed that ethanol extract of *T. polium* has an antioxidant activity with IC_{50} = 15.1. For the FRAP assay, we found that ferric reducing ability of *T. polium* extract is high (6.41 µg TEAC/g). These results proved that *T. polium* extract has high antioxidant properties due to the high total phenols and flavonoids. Such phenolic compounds were reported by many studies to be a strong antioxidants and radical scavenging agents [20-23].

Until now, there is no anti-inflammatory activity of *T. polium* plant from Palestine and this fact motivated us to give more insight into this activity. Ethanol was used in this work to extract phytochemicals from this plant as it combines polar and medium polarity solvent. Present results showed that the *T. polium* ethanoic extract has strong anti-inflammatory effect. This work agrees with previous study of Rahmouni *et al.* [24] and Amraei *et al.* [25]. Our work was unique in its investigation by using ethanolic extract exposed to LPS stimulated poly morphonuclear cells (PMNCs). The concentrations of the extract were gradually increased to investigate both its cytotoxicity as well as its anti-inflammatory effect. The ethanolic extract of *T. polium* did not show any significant cytotoxicity.

Moreover, an increase in the extract concentration showed a significant decrease in TNF-α concentration indicating its strong anti-inflammatory effect. However, it appears that anti-inflammatory effect of the extract may related to the presence of flavonoids and phenolics in the plant [24,25].

Conclusion

In the present study, *Teucrium polium* leaves were screened for their potential antioxidant and anti-inflammatory activities. Based on the results, it could be concluded that *T. polium* exhibited different bioactivities, which supports their potential use as therapeutic medicinal plant having strong antioxidant and anti-inflammatory effects.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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