

Antioxidant status of thyme and rosemary leaf powders and their effect on lipid oxidation of minced meat during cold storage

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Abstract

This study examined the antioxidant capacities of rosemary and thyme leaf powders and their impact on lipid oxidation in minced beef during refrigerated storage. Five types of minced beef were prepared: Control (meat without additives), TLP 0.2 (meat + 0.2% thyme leaf powder), TLP 0.4 (meat + 0.4% thyme leaf powder), RLP 0.2 (meat + 0.2% rosemary leaf powder) and RLP 0.4 (Meat + 0.4% rosemary leaf powder), and stored at 4°C for seven days. Results revealed that RLP exhibited higher ($P < 0.05$) phenol (398.75 mg GAE/g), flavonoid (172.30 mg QE/g) and vitamin C (82.05 mg/g) contents than TLP (297.61 mgGAE/g, 65.72 mgQE/g and 52.56 mg/g, respectively). Extracts of RLP demonstrated higher ($P < 0.05$) antiradical activity than TLP. During day zero of storage, there was no significant effect of leaf powders on the minced beef. However, on day three, both RLP and TLP at 0.2% and 0.4% significantly reduced lipid oxidation in the minced beef than the control group. Also on day seven, the lipid oxidation of RLP and TLP minced beef was greatly reduced ($P > 0.05$) compared to the control group. Thus, application of RLP and TLP could serve as effective preservatives, inhibiting lipid oxidation in meat products.

Introduction

Beef is one of the most widely consumed meat sources, which is appreciated for its taste, nutritional value, and versatility in culinary applications. Nutritionally, beef contains high protein content (22%), moderate fat content (about 10%) with an appropriate ratio of n-6/n-3 polyunsaturated fatty acids (PUFAs) and essential minerals including phosphorus, potassium, calcium, and sodium as well as vitamins such as vitamin E, thiamin, and riboflavin (Meng et al., 2022). The quality of beef products is related to many factors, such as muscle parts, ingredients, processing, and storage techniques (Meng et al., 2022). Specifically, the quality and shelf life of minced beef are notably impacted by lipid oxidation, a complex chemical process that occurs throughout processing and storage, significantly influencing its overall condition (Falowo et al., 2017).

The onset of lipid oxidation initiates as the fats within the beef react with oxygen, ultimately resulting in the degradation of sensory characteristics such as taste, color, and aroma (Falowo et al., 2014). Furthermore, this process has the potential to impact the meat's nutritional value by diminishing essential fatty acids and producing harmful compounds (Falowo et al., 2014).

The pursuit to mitigate this oxidative process has led to a growing interest in employing natural antioxidants as a promising alternative to synthetic counterparts, which have been associated with adverse effects on consumer health (Falowo et al., 2014; Lourenço et al., 2019; Jaworska et al., 2021). Natural antioxidants are naturally occurring additives known for their antioxidative properties, capable of potentially retarding or inhibiting the oxidative breakdown of lipids

in meat products, thereby sustaining their quality over an extended duration (Falowo et al., 2014; Horbańczuk et al., 2019). Some of the most commonly used natural antioxidants are tocopherols, ascorbic acid, herbs, spices, and their extracts (Lourenço et al., 2019). Among the herbs and spices that have been used for centuries to prepare meat products due to their inherent palatable taste and aroma are rosemary and thyme leaf powders (Essid et al., 2018; Ricardo-Rodrigues et al., 2024).

Rosemary (*Rosmarinus officinalis*), an aromatic plant, is one of the species widely used around the world in the food industry. The plant has been characterized by high preservative, antioxidant, antifungal, and antimicrobial activity, and its utilization in food products has demonstrated efficacy in curbing lipid oxidation (Lorenzo et al., 2021). The efficacy of rosemary plant to curb lipid oxidation has been attributed to its inherent bioactive compounds, such as carnosic acid, carnosol, and rosmarinic acid, which scavenge free radicals, chelate metal ions, and inhibit oxidative chain reactions (Amaral et al., 2018). Similarly, thyme (*Thymus vulgaris* L.), an herbaceous perennial herb from the genus *Thymus*, is another medicinal plant widely used as food preservatives and condiment as it provides high antioxidant and antibacterial properties. Originating in the Mediterranean and now cultivated worldwide, thyme leaf is commonly integrated into rabbit, boar, and lamb meats to augment their palatability, enrich sensory qualities and prevent lipid oxidation (Nieto, 2020). Thyme contains monoterpene phenols, including carvacrol, thymol, and p-cymene, as well as other monoterpenes such as α -pinene, 1,8-cineole, camphor, linalool, and borneol. These compounds function as free radical scavengers, metal ion chelators, and inhibitors of oxidative enzymes (Nieto, 2020). Different research has indicated that incorporating plant leaf powders into meat products can enrich their nutritional profile by adding more antioxidants, which can positively benefit consumers' health upon consumption (Jaworska et al., 2021; Mashau et al., 2021). However, there remains a paucity of information regarding the efficacy of rosemary and thyme leaf powders in mitigating lipid oxidation in meat products. To our knowledge the preservative effect of rosemary and thyme leaf powders in beef products as potential antioxidants has not been studied. Therefore, the aim of this study was to examine the antioxidant properties of thyme and rosemary leaf powders and evaluate their impact on minimizing lipid oxidation in minced beef during refrigerated storage.

Materials and Methods

Materials and plant extract preparation

Processed thyme and rosemary leaf powders were procured from a reputable supermarket in Akure, Nigeria, and securely stored in a cool, dry environment until their utilization. Fresh beef was purchased from a local processing meat shop in Akungba-Akoko, Nigeria.

All chemicals and reagents were purchased from Pascal Scientific Limited (Akure, Nigeria) and Sigma Chemicals (Steinheim, Germany).

For extract preparation, five grams of each leaf powder were individually placed in glass thimbles. A total of 200 mL of distilled water was employed as the extraction solvent. The mixture underwent heating on a hot plate, maintaining a temperature range of 30-40°C, while being continuously stirred for 20 min. Following the extraction, the extract was filtered using Whatman No. 1 filter paper and then concentrated to a dry state using a rotary evaporator. The concentrated extracts were lyophilized with a freeze-drier and the dried extracts were used for the determination of the antioxidant content and activity.

Determination of antioxidant content of plant material

Total phenolic content

The total phenolic compound concentration in each leaf extract was assessed spectrometrically through the Folin–Ciocalteu method (Wolfe et al., 2003), employing gallic acid as a standard for constructing a calibration curve. A volume of 1 mL of leaf extract (10 g/L) was combined with 5 mL of Folin–Ciocalteu reagent and 4 mL (75 g/L) of sodium carbonate. After an incubation period of 1 h, the absorbance of the reaction mixture was measured at 765 nm against a methanol blank, utilizing a Shimadzu UV-1700 UV/vis spectrophotometer (Tokyo, Japan). Results were quantified in milligrams of gallic acid equivalent (GAE) per gram of extract, determined based on the established gallic acid calibration curve within a linearity range of 1–10 μ g/mL.

Total flavonoids content

The determination of total flavonoid content in each aqueous extract followed the colorimetric assay detailed by Bao (2005). Briefly, 0.2 mL of each extract was combined with 0.3 mL of 5% NaNO₃ at the start. After 5 min, 0.6 mL of 10% AlCl₃ was introduced, followed by the addition of 2 mL of 1M NaOH after 6 min. Subsequently, 2.1 mL of distilled water was added to the mixture. The absorbance was measured at 510 nm against the reagent blank, and the flavonoid content was quantified in mg QE equivalent.

Vitamin C (Ascorbic acid) content

The determination of vitamin C (vit C) content in the leaf samples followed the method outlined by Benderitter et al. (1998). A solution composed of 75 μ L DNPH (2 g dinitrophenyl hydrazine, 270 mg copper sulfate (CuSO₄.52O), and 230 mg thiourea in 100 mL of 5 ml/L H₂SO₄) was added to a mixture comprising 500 μ L of extract (prepared by diluting 300 μ L of the extract with 100 μ L of 13.3% trichloroacetic acid and water). Following this, the reaction mixture underwent incubation at 37°C for 3 h. Subsequently, 0.5 mL of 65% H₂SO₄ (v/v) was added to the medium, and the

absorbance was measured at 520 nm using a UV spectrophotometer. The vit C content of the leaf powder was quantified using ascorbic acid as the reference standard.

Determination of antioxidant activity

DPPH (1,1-diphenyl-2-picrylhydrazyl free radical)

The assessment of the aqueous extracts' free radical scavenging capacity against DPPH (1,1-diphenyl-2-picrylhydrazyl) followed the methodology described by [Gyamfi et al. \(1999\)](#). A volume of 1 mL of the extract was combined with an equal volume of a 0.4 mM methanolic solution of DPPH. The resultant mixture was shielded from light and left to stand for 30 min before measuring the absorbance at 516 nm.

ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid)

The determination of ABTS free radical scavenging activity in plant extracts was conducted following the method outlined by [Re et al. \(1999\)](#). ABTS radicals were generated by incubating an ABTS solution (7 nmol/L) with $K_2S_2O_8$ (2.45 mmol/L, final concentration) in darkness for 16 h until the absorbance at 734 nm was adjusted to 0.700 using ethanol. The extracts were diluted at a ratio of 1:10. Subsequently, 0.2 mL aliquots from each extract were added to 2.0 mL of the ABTS solution, and the absorbance was measured after 15 min at 734 nm using a UV-visible spectrophotometer. The ABTS radical scavenging capacity of the extracts was evaluated by comparison with the reference standard trolox."

Preparation of minced beef

Three kg of boneless fresh beef were purchased from a processing meat shop in Akungba-Akoko, Ondo state, Nigeria. The visible connective tissues and fat were removed through trimming, and the meat was cut into small cubes and minced using a meat grinder. Five formulations of the minced beef were prepared: Control (meat sample without additives), TLP 0.2 (minced beef with 0.2% thyme leaf powder), TLP 0.4 (minced beef with 0.4% thyme leaf powder), RLP 0.2 (minced beef with 0.2% rosemary leaf powder) and RLP 0.4 (minced beef with 0.4% rosemary leaf powder). The meat samples (100g) were packed in polyethylene plastic bags and stored for zero, three, and seven days in a refrigerator at 4°C for analysis of lipid oxidation.

Lipid oxidation analysis

The lipid oxidation of the meat samples was assessed at zero, three, and seven days during cold storage at 4°C by analysis of Thiobarbituric acid reactive substances (TBARS) based on the method described by [Xia et al. \(2012\)](#). TBARS values were measured and expressed as mg of malondialdehyde (MDA) per kg beef sample and then calculated as follows:

$$\text{TBARS (mg/kg)} = A532/Ws \times 9.48$$

where A532 represents the absorbance of the solution measured at 532 nm, Ws is the sample weight (g), and "9.48" is a constant originating from the dilution factor and the molar extinction coefficient ($1.52 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$) of the Thiobarbituric acid (TBA) reactive product.

Statistical analysis

Data obtained on antioxidant contents and activity of the plant leaf powders were analyzed using the T-tests package of SPSS (version 20 statistical software). The lipid oxidation (TBARS) values were subjected to one-way ANOVA using SPSS version 20 statistical software. Differences between means were considered statistically significant at $P < 0.05$. All analyses were done in triplicate. While the TBARS analysis for beef samples was carried out in three replicates per each treatment and storage day.

Results and Discussion

The antioxidant contents (phenol, flavonoid, and vit C) of the plant leaf extract in this study are presented in Figures 1-3. The results revealed that RLP exhibited significantly higher ($P < 0.05$) phenol (398.75 mg GAE/g) and flavonoid (172.30 mg QE/g) contents than TLP (297.61 mg GAE/g and 65.72 mg QE/g, respectively). The analysis of phenol and flavonoid content in the leaf extracts showed that both RLP and TLP are rich in antioxidants. Nevertheless, the notably higher levels of phenols and flavonoids detected in RLP could be indicating a greater antioxidant potential than TLP. Phenols and flavonoids are known secondary metabolites that are responsible for neutralizing free radicals and reducing oxidative stress based on their ability to donate hydrogen atoms to free radicals ([Aryal et al., 2019](#); [Shahar et al., 2023](#)). In addition, these molecules have been reported to possess specific health-promoting benefits such as antioxidant, antimicrobial, anticancer, anti-inflammatory, antidiabetic, and hypocholesterolemic properties when used in biological systems ([Albuquerque et al., 2021](#); [Falowo, 2022](#); [Shahar et al., 2023](#)). Moreover, the total phenol content determined in this study surpassed that reported by [Hendrawan et al. \(2019\)](#) and [Zeili et al. \(2024\)](#) for aqueous extracts of thyme and rosemary leaves. Conversely, the flavonoid content was lower than the values reported by [Zeili et al. \(2024\)](#) and [Hendrawan et al. \(2019\)](#) for similar extracts. These discrepancies in phenol and flavonoid content might be attributed to variations in analytical methodologies, diverse plant cultivars utilized, parts of the plant used for analysis, and the stage of plant maturity ([Falowo, 2022](#)).

The vit C content of the leaf extract was significantly lower in TLP (52.56 mg/g) than RLP (82.05 mg/g). Vit C is a natural antioxidant that plays a vital role in immune function, collagen synthesis, and overall health of the body ([Dumbrava et al., 2012](#)). It is used to inhibit lipid oxidation and safeguard the sensory and

nutritional characteristics of the food products (Yin et al., 2022). The amount of vit C reported in this study was higher than those reported by Dumbraava et al. (2012) and Shahar et al. (2023) for rosemary and thyme leaf powder.

The results of the antioxidant (DPPH, 1,1-diphenyl-2-picrylhydrazyl, and ABTS, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid) activities of the plant extracts are presented in Figures 4-5. The extracts of RLP exhibited stronger ($P < 0.05$) antioxidant activity than that TLP. Specifically, the percentage inhibition of DPPH radicals for RLP and TLP were 85.05% and 78.57%, respectively, while that of ABTS radicals was 98.28% for RLP and 95.65% for TLP. The higher DPPH and ABTS free radical scavenging ability of RLP observed in this study could be attributed to the abundant presence of phenolic and flavonoid compounds inherent in the extract (as shown in Figures 1-3). Previous studies have consistently demonstrated a strong linear correlation between DPPH/ABTS radical scavenging capacity and the phenolic/flavonoid content in plant leaf extracts (Rajurkar and Hande, 2011; Aryal et al., 2019; Oyeyinka and Afolayan, 2020; Trinh et al., 2020). This correlation underscores that the presence of inherent phenolic and flavonoid compounds is strongly responsible for the antioxidant activity of the plant leaf extracts (Aryal et al., 2019).

Table 1 shows the effect of thyme and rosemary leaf extracts on lipid oxidation (TBARS) of minced beef during cold storage (4°C) at days zero, three, and seven. The level of lipid oxidation increased progressively with an increase in storage days. On day zero, the addition of the RLP and TLP at 0.2 and 0.4% did not exhibit a significant effect on TBARS values across the treatments. However, on day three, the addition of RLP and TLP significantly reduced ($P < 0.05$) TBARS values in the minced beef compared to the control group. On day seven, although the reduction was not statistically significant ($P < 0.05$), the inclusion of RLP and TLP led to lower TBARS values than the control group. The decline in TBARS values observed in minced beef treated with RLP and TLP could be attributed to their inherent phenol and flavonoid content, along with their antioxidant activity. Numerous studies have highlighted a strong correlation between the antioxidant content of plant leaf powders/extracts and their efficacy in reducing TBARS values in meat products during cold storage (Falowo et al., 2017; Mashau et al., 2021). This result aligns with findings from Jaworska et al. (2021) and Mashau et al. (2021), who reported a significant reduction in lipid oxidation in meat products treated with oregano and *Moringa oleifera* leaf powders, respectively, compared to control groups. Overall, the incorporation of RLP in minced beef yielded lower TBARS values than TLP. This disparity could be linked to the higher antioxidant content and activity observed in the RLP than TLP.

Conclusion

The study highlights the richness of antioxidant content (phenol, flavonoid, and vit C) and significant antioxidant activities present in both rosemary and thyme leaf powders. Notably, the antioxidant content and capacity of rosemary leaf powder surpass those of thyme leaf powder. Moreover, both rosemary and thyme leaf powders, when applied at concentrations of 0.2% and 0.4%, demonstrate the potential in mitigating lipid oxidation in meat products during cold storage. Also these findings indicate that RLP and TLP are promising natural sources of antioxidants, offering a viable alternative to synthetic antioxidants in the meat industry.

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Table 1. Effect of rosemary and thyme leaf powders on lipid oxidation (mg MDA/kg) of minced beef during cold storage.

Storage days	Control	TLP 0.2%	RLP 0.2%	TLP 0.4%	RLP 0.4%	SEM	<i>P value</i>
Day 0	0.32	0.40	0.33	0.34	0.39	0.46	0.27
Day 3	0.79 ^a	0.80 ^a	0.54 ^{ab}	0.57 ^{ab}	0.34 ^b	0.63	0.05
Day 7	1.40	1.12	1.01	0.93	0.75	0.09	0.22

Means within a row with different letters and significantly different ($P < 0.05$). SEM Standard error. TLP: Thyme leaf powder, RLP: Rosemary leaf powder.

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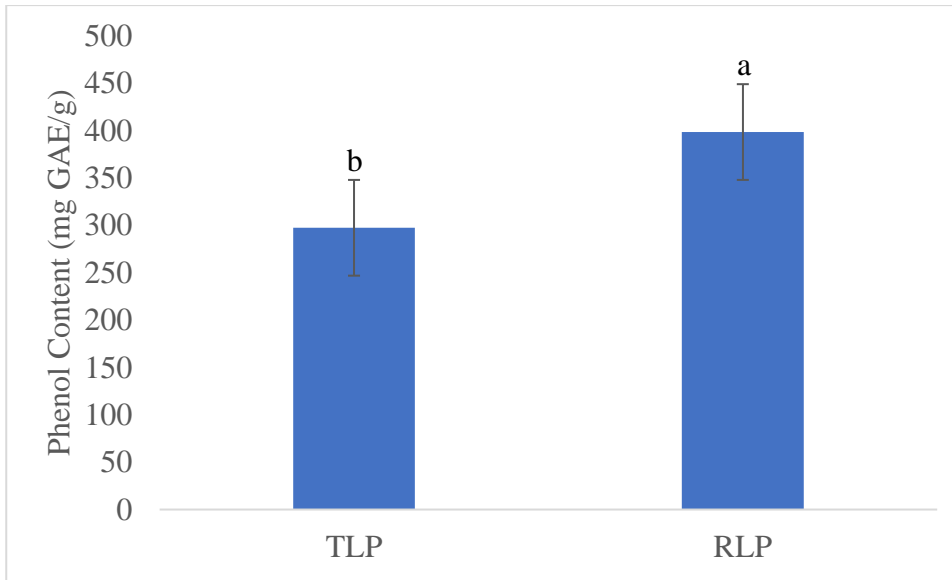


Figure 1. Phenolic content of the plant leaf extracts. TLP: Thyme leaf powder, RLP: Rosemary leaf powder. Means within the figure with different letters are significantly different ($P < 0.05$).

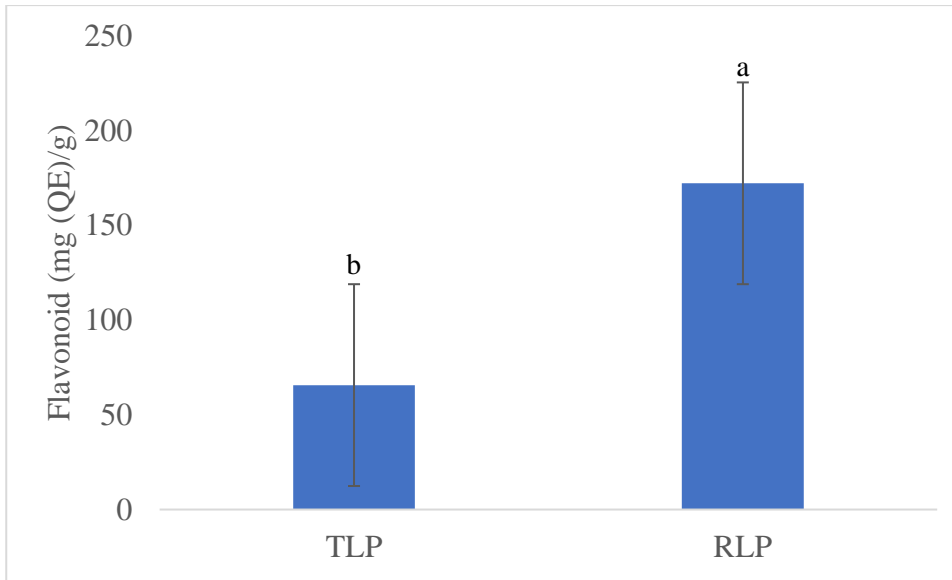


Figure 2. Flavonoid content of the plant leaf extracts. TLP: Thyme leaf powder, RLP: Rosemary leaf powder. Means within the figure with different letters are significantly different ($P < 0.05$).

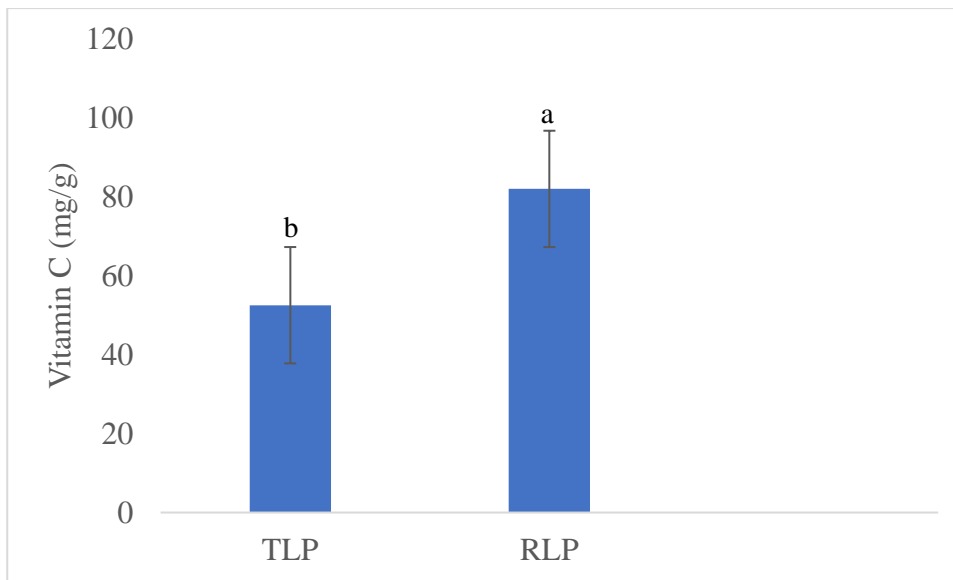


Figure 3. Vitamin C content of the leaf powders. TLP: Thyme leaf powder, RLP: Rosemary leaf powder. Means within the figure with different letters are significantly different ($P < 0.05$).

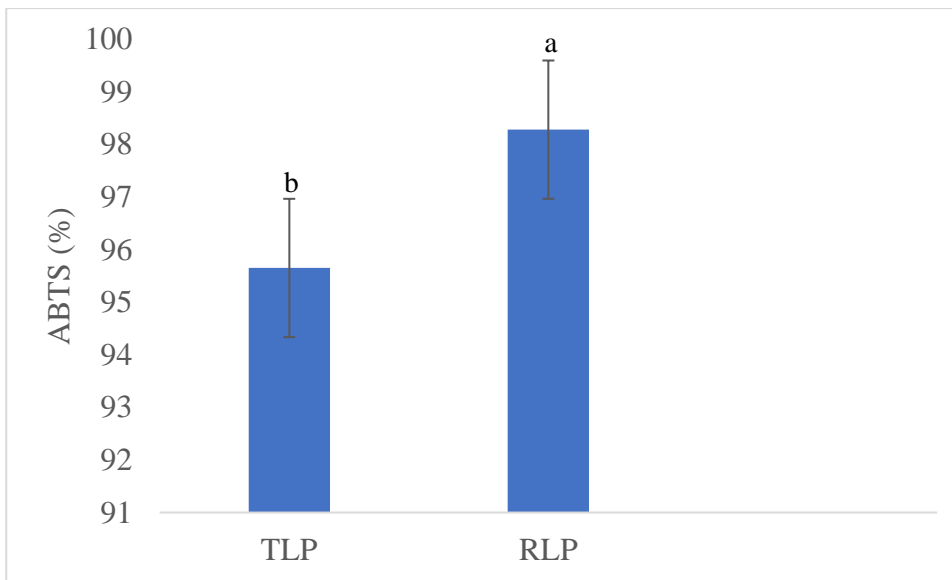


Figure 4. Antioxidant activity (2, 2-azino- bis-3-ethylbenzothiazoline-6-sulfonic acid, ABST) of the plant leaf extracts. TLP: Thyme leaf powder, RLP: Rosemary leaf powder. Means within the figure with different letters are significantly different ($P < 0.05$).

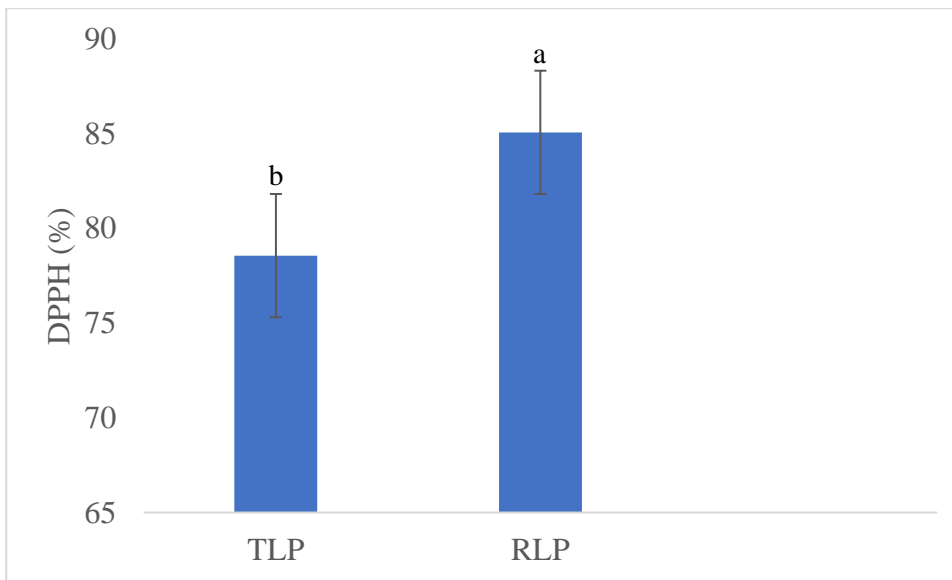


Figure 5. Antioxidant activity (2, 2-Diphenyl-2-picrylhydrazyl, DPPH) of the plant leaf extracts. TLP: Thyme leaf powder, RLP: Rosemary leaf powder. Means within the figure with different letters are significantly different ($P < 0.05$).