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FULL PAPER

In vitro antioxidant activity of *Lippia adoensis* Var. Koseret, *Thymus schimperi* Ronniger and *Rosmarinus officinalis Leaf* extracts and their effects on oxidative stability of ground raw beef meat during refrigeration storage

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Abstract

Rosmarinus officinalis,Thymus schimperi Ronniger, and *Lippia adoensis* var. koseret, locally known as *Rosemary*, *Tosign*, and *Koseret* respectively are widely used dietary herbs in Ethiopia. In this study, the antioxidant activity and effect of *Rosemary*, *Tosign*, and *Koseret*ethanol (80% v/v) extract on lipid oxidation of ground beef during storage were studied. Folin-Ciocalteu and aluminium chloride were used to determine total phenolic content (TPC) and total flavonoid content (TFC), respectively. Antioxidant activities of herbs were evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, ferric reducing power and iron chelating activities. The peroxide and thiobarbituric acid reactive substances assays were used to evaluate the ability of the extracts to prevent lipid peroxidation in ground beef stored at 4°C. *Tosign* extract had highest TPC (70.93±1.53 mg GAE/g) and TFC (16.94±0.12 mg CE/g). Also, *Tosign* had the strongest effect of DPPH scavenging (IC_{50} = 33.35±1.56µg/mL) and ferric reducing power (IC_{50} = 175.71±1.03 µg/mL). But the strongest iron-chelating activity was observed in *Rosemary* extract (IC_{50} = 160.24±2.55 µg/mL). Minimum peroxide value (0.185±0.09 milli equivalent peroxide/kg of beef) and the highest thiobarbituric acid reactive substances (80.20±20%) were recorded for ground beef treated with *Rosemary* at the end of storage days. In conclusion, these herbs are very effective antioxidants comparable to butylated hydroxytoluene. Thus, they could be a good substitution to synthetic antioxidants used in food preservation.

1. Introduction

Meat and meat products play an important role in providing energy, high-quality, readily digestible protein with all essential amino acids and other absorbable micronutrients which are needed for human growth, cell functioning and sound health (De Smet, 2012; Mourouti *et al*., 2015). It is also a rich source of lipids which are important for the flavour and aroma profile of meats and contribute to tenderness and juiciness. Lipid oxidation is one of the primary causes of deterioration in the quality of meat during storage, leading to the development of off -flavour, as well as reduced nutritional quality, shelf-life stability and acceptability. It is accelerated by the processes of grinding, chopping and cooking (Amaral *et al*., 2018)

Synthetic and natural antioxidants have been successfully used to block or delay the oxidation process in meat. In addition to their ability to increase lipid stability, antioxidants added to foods may have the ability to reduce the risk of various diseases related to the production of free radicals (Diplock, 1994). However, the use of synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) has been restricted because of possible health risks, toxicity and carcinogenic (Naveena *et al*., 2008). In this regard, there is an increasing interest in the identification and development of natural antioxidants from herbs and spices such as rosemary, oregano, broccoli, sage, black cumin, thyme or turmeric, as well as vegetables and fruits such as olives, pomegranate, grapes and berries (Naveena *et al.*, 2008; Shahidi and Ambigaipalan, 2015).

Lippia adoensis var. Koseret, locally known as *Koseret* and *Thymus schimperi* Ronniger known as '*Tosign'* are endemic aromatic herbs to Ethiopia commonly used by Ethiopian people for flavoring and

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preserving food and also as traditional medicine (Aziz and Karboune, 2018). The dried leaves of *L. adoensis* var. Koseret are used as one of the ingredients in the preparation of traditional spiced butter. The special taste and flavor of the Gurage kitfo (minced meat with spiced butter) are attributed to essential oil imparted by the leaves (Nigist and Sebsebe, 2009; Engeda *et al*., 2019). The dried leaves of *T. shimperi* have been used to flavor tea, coffee, food and also boiled as a tea substitute and are believed to be good for diabetic patients (Nigist and Sebsebe, 2009). Rosemary is a popular dietary herb and evergreen plant belonging to the Lamiaceae family, and its extracts have a potential antioxidant activity (Mena *et al*., 2016). The antioxidant activity of *Rosemary* extracts has been associated with the presence of several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol (Borrás-Linares *et al*., 2014). According to Karre *et al*. (2013), it is widely used as a preservative in meat and poultry products and also has antimicrobial and antifungal effects. Different authors also reported on *Rosemary* extracts for the prevention of autoxidation in sunflower oil (Chen *et al*., 2014),pork-based products (Lara *et al*., 2011) and other types of foods such as sausages (Georgantelis *et al*., 2007) and chicken nuggets (Teruel *et al*., 2015). Oral toxicity of hydro-alcoholic extract of leaves *R. officinalis* was found to be safe at the highest dose level of 1000 mg/kg for 28 days of oral administration (Salokhe *et al*., 2020). Similarly, the research work by Destaw *et al*. (2017) on *T. schimperi* leaf extracts in mice showed no significant signs of toxicity at doses of 100 mg and 200 mg/ kg body.

Many studies have been conducted on bioactive phytochemicals and their effects on human health. In particular, researchers have focused on food preservative activities of naturally occurring antioxidants. However, there are limited research efforts so far conducted to explore the evaluation of the antioxidant activity of selected endemic spices and herbs on the lipid stability of meat and meat products in Ethiopia (Engeda *et al*., 2015). Therefore, the main objective of the current study was to compare the potential role of natural antioxidants such as *Rosemary*, *Tosign* and *Koseret* on delaying lipid oxidations of ground raw beef meat.

2. Materials and methods

2.1 Chemicals

Gallic acid, butylated hydroxytoluene (BHT), Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), catechin, ferrozine, L-ascorbic acid, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), sodium carbonate, thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other chemicals and solvents used in this experiment were of analytical grade.

2.2 Sample preparation and extraction

Rosemary and *Koseret* were collected from the Ethiopian Institute of Agriculture, Wondo Genet Research Center, in the wintertime. Whereas *Tosign,* was collected from the highland of Bale National Park, Bale zone, South East Ethiopia. Approximately 10 g of powdered dried herbs were mixed into 100 mL of 80% (v/v) ethanol for 8 hrs in an enclosed beaker with constant shaking (Odey *et al.*, 2012). Thereafter, each extract was filtered and concentrated in a rotary evaporator (Buchi, 3000 series, Switzerland) under vacuum at 40°C. The extracts were sealed in a polyethylene bag container and stored at 4°C until further investigation.

2.3 Beef meat treatments

The beef was collected from Hawassa City abattoirs and transported to the laboratory using ice bags. The beef was free of bones, cartilage, exposed lymph glands, heavy connective tissue and the tendon's ends of shanks. It was cut into small pieces to grind with a diameter of 0.2 mm size by an electrical grinder (model of NIMA.NM-786). Ground beef was divided into five batches. *Rosemary*, *Tosign* and *Koseret* extracts (50 mL,1 mg/ mL) were added to three batches of ground beef (300 g), and the remaining batches, in the absence of the extract and in the presence of BHT, were used as negative and positive controls, respectively. Then samples were mixed and packed in low-density permeable polyethylene bags (Lloha and Mara, 2013). Then, they were stored in the refrigerator at 4^{o}C for fifteen days.

2.4 Total phenolic content

The total phenolic content of extracts was determined using the Folin–Ciocalteau reagent as described by Engeda *et al*. (2015) with slight modification. For 0.1 mL of the extracts (1 mg/mL), 1 mL of diluted Folin-Ciocalteu reagent (1:10) was added and allowed to stand for 5 mins. Then, 1 mL of sodium carbonate was added and incubated for 90 min at room temperature. The absorbance of the resulting blue color was measured at 765 nm with a UV- visible spectrophotometer (JENWAY, 96500, UK). The total phenolic content was estimated from gallic acid (1-100 µg/mL) calibration curve $y = 0.015x + 0.081$, $R^2 = 0.991$ ($p < 0.001$) and the results were expressed as milligram of gallic acid equivalent per gram of dried extract (mg GAE/g) using the equation, $C = [(c \times V)/m]$.

Where C = total phenolic contents (mg GAE/g dried extract), c = concentration established from gallic acid calibration curve (µg/mL), V = volume of extract in

milliliter, m = the weight of dried extract in gram.

2.5 Total flavonoid content

The total flavonoid content of the extracts was determined according to Ayoola *et al*. (2008) with slight modification. The extracts (1 mL, 1 mg/mL) were diluted with 1.25 mL distilled water and 75 µL of 5% $NaNO_2$ was added. After 5 mins, 150 µL of 10% $AlCl_3$ was added. After 6 mins, 1 mL NaOH was added. Then immediately, the absorbance was measured at 510 nm. The TFC was determined using a standard curve (y = 0.024x + 0.112, R^2 = 0.99) of catechin and values were expressed as milligram of catechin equivalent per gram of dried extract (mg CE/g), using the equation, C = [(c × V)/m].

Where C = total flavonoid contents (mg CE/g), c = concentration established from the catehin calibration curve (µg/mL), V = volume of dried extract in milliliter, m = the weight of dried extract in gram.

2.6 Antioxidant activities

2.6.1 DPPH scavenging activity

DPPH free radical scavenging activity was determined as described by Jundi *et al*. (2021). The absorbance rates of the DPPH in the presence of herb extracts and butylated hydroxytoluene (BHT) as reference standards were measured at 520 nm. The ability to scavenge the DPPH radical was calculated as:

$$\text{DPPH Scavenging capacity (\%)} = (1 - \frac{\text{Abs sample}}{\text{Abs control}}) \times 100$$

Where Abs is the absorbance of the sample and Abscontrol is the absorbance of DPPH in the absence of the sample extract. The scavenging activity of the extracts was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/mL) of extracts that scavenges the DPPH radical by 50%.

2.6.2 Ferrous chelating activity

The ferrous chelating activity was determined using Ebrahimzadeh *et al*. (2008) method with slight modification. The absorbance of the solution was measured at 562 nm, and ethylenediamine tetraactic acetic acid (EDTA) was used as a control. The inhibition percentage of ferrozine-Fe^{2+} complex formation was calculated by using the formula:

$$\text{Iron chelate activities (\%)} = (1 - \frac{\text{Abs sample}}{\text{Abscontrol}}) \times 100$$

Where Abs sample is the absorbance ferrozine-Fe^{2+} complex in the presence of sample extract and Abs control is the absorbance of ferrozine-Fe^{2+} complex in the absence of sample extract.

2.6.3 Ferric reducing power

The presence of antioxidants in the extract causes the reduction of the yellow ferric/ferricyanide complex to the ferrous form, which can be monitored by measuring the formation of Perl's Prussian blue at 700 nm was determined according to the method reported by Amarowicz *et al*. (2004) with some modification. Different concentrations of solution of 1 mL (100 mg/mL) in methanol were mixed with 2.5 mL potassium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. Then the mixture was incubated at 50°C for 20 min. Then 2.5 mL trichloroacetic acid of 10% was added to the mixture. Finally, 2.5 mL of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL $FeCl_3$ (0.1%). Absorbance was measured to determine the amount of ferrocyanide (Perl's Prussian blue) at 700 nm against methanol as a blank using a double beam visible spectrophotometer. Higher absorbance (A_{700}) represents a stronger reducing power. IC_{50} values (µg/mL) were calculated by plotting the absorbance against the corresponding sample concentration, representing the effective concentration at which the absorbance was 0.5 for reducing power. Ascorbic acid was used as a reference compound.

2.7 Peroxide value

The peroxide value (POV) of beef was determined by the AOAC (1999). The beef sample (3 g) was weighed in a 250 mL glass stopped Erlenmeyer flask and heated in a water bath at 60°C for 3 mins to melt the fat, then thoroughly agitated for 3 mins with 30 mL acetic acid–chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered using filter paper to remove meat particles. A saturated potassium iodide solution (0.5 mL) was added to the filtrate, which was transferred into the flask. Approximately 0.5 mL of 1% starch solution (indicator) was added, and titration was run against a standard solution of sodium thiosulfate (N = 0.01) until the blue color just disappeared. Then Peroxide value (POV) was calculated by using the following equation:

$$\text{Peroxide value (meq/kg)} = S \times N \times \frac{1000}{W\ (g)}$$

Where S is the volume of titration (mL), N is the normality of sodium thiosulfate solution, and W is the sample weight (g)

2.8 Thiobarbituric acid reactive substances assay

The Thiobarbituric acid reactive substances (TBARS) value was determined according to Jayathilakan *et al*. (2007) using a UV-Vis spectrophotometer. From the previously prepared sample used to measure the peroxide value, 1 mL was added to

the TBA reagent (1 mL of 15% (w/v) TCA and 2 mL of 0.375% (w/v), TBA in 0.25 M HCl). The reaction mixtures were then kept in a water bath at 85°C for 15 mins. After cooling, it was centrifuged at 3000 rpm for 20 mins, and the absorbance of the supernatant was then measured at 532 nm. The antioxidant activity was calculated by a percentage of inhibition, % Inhibition = $100 - [A_1/A_o] \times 100$.

Where A_o is the absorbance of the control and A_1 is the absorbance of the sample extracts.

2.9 *Statistical analysis*

The data were subjected to ANOVA (analysis of variance) by using SAS version 9.2. The IC_{50} value was determined by using origin 8. Mean separation was conducted using Duncan's multiple range tests at $p < 0.05$. The data were analyzed in triplicate, and the results are expressed as the mean values ± standard deviation (SD).

3. Results and discussion

3.1 Total phenolic and flavonoid contents

The total phenolic content of the three ethanol extracts varied from 43.99 to 70.93 mg GAE/g (Table 1). *Tosign* extract had significantly ($p < 0.05$) the highest total phenolic content compared to *Rosemary* and *Koseret* extract. TPC of *Tosign* obtained from this study was higher than that of the sample collected from the central highlands of Ethiopia (Engeda *et al*., 2015). Similarly, the *Tosign* extract was the richest source of TFC ($p < 0.05$) and decreased in the order of *Tosign* > *Rosemary* > *Koseret* (Table 1). The TPC and TFC of the present study were lower than that of *Tosign* and *Koseret* reported by Engeda *et al*. (2015) also, the TPC of rosemary was lower than that reported by Turan. (2014). This variation may be because of geographical location or soil type (Oney-Montalvo *et al*., 2020).

Table 1. Total phenolic and flavonoid content of *Rosemary, Tosign* and *Koseret* extracts.

Sample	TPC (mg GAE/g)	TFC (mg CAE/g)
Rosemary	50.23±0.04^{b}	14.06±2.20^{b}
Koseret	43.99±1.22^{c}	7.20±0.56^{c}
Tosign	70.93±1.53^{a}	16.94±0.12^{a}

Values are presented as mean±SD (n = 3) from triplicate experiments. Values with different superscript within the same column are significantly different at $p < 0.05$. TPC: total phenolic content expressed in terms of gallic acid equivalent, TFC: total flavonoid content expressed in terms of catechin equivalent.

3.2 Antioxidant activities

3.2.1 DPPH scavenging activity

The DPPH scavenging activities of *Rosemary*, *Tosign* and *Koseret* leave extracts are shown in Figure 1. As the concentration of the sample increased, the percentage of inhibition of DPPH radical also increased (Labiad *et al*., 2017). The DPPH scavenging activity was concentration-dependent (Figure 1). The IC_{50} values of all the extracts were calculated from plotted graph of percentage scavenging activity against the concentration of the extracts (Table 2). The lower the IC_{50} value, the higher the scavenging potential. Among all extracts, *Tosign* extracts exhibited the strongest DPPH radical scavenging activity with IC_{50} of 33.33 µg/mL followed by *Rosemary* (47.16 µg/mL) and *Koseret* (68.06 µg/mL) (Table 2). The IC_{50} values of *Koseret* and *Rosemary* were significantly different ($p < 0.05$). Also, these values were significantly weaker ($p < 0.05$) than that of *Tosign* extract. Whereas, *Tosign* has no significant scavenging difference ($p > 0.05$) with ascorbic acid. The DPPH scavenging activities of *Tosign* and *Koseret* in this study were weaker than that of samples collected from the central highland of Ethiopia (Engeda *et al*., 2015; Engeda *et al*., 2020). This difference may be because of geographical location, soil type and season of harvesting (Nateqi and Mirghazanfari, 2018; Gedikoğlu *et al*., 2019; Oney-Montalvo *et al*., 2020).

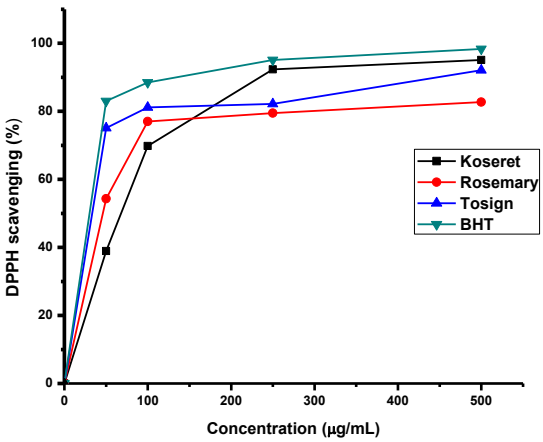

Figure 1. DPPH radical scavenging activity of *Rosemary*, *Tosign,* and *Koseret* ethanol extracts and control (BHT). Values are the average of triplicate measurements (mean±SD).

Table 2. IC_{50} values (µg/mL) of ethanol extracts of *Rosemary*, *Tosign* and *Koseret*

Sample	DPPH scavenging	Ferric reducing power	Iron chelating
Rosemary	47.16±0.86^{b}	318.44±6.9^{c}	160.24±2.55^{b}
Koseret	68.06±5.70^{c}	338.6±9.76^{c}	196.27±5.61^{c}
Tosign	33.35±1.56^{a}	175.71±1.03^{b}	176.60±3.63^{b}
Ascorbic acid	30.36±0.26^{a}	64.76±8.2^{a}	-
EDTA	-	-	55.43±1.65^{a}

Values are presented as mean±SD (n = 3). Values with different superscript within the same row are significantly different at $p < 0.05$. EDTA: ethylenediamine tetraactic acetic acid, excellent chelator for ferrous ions.

3.2.2 Ferric reducing power

Fe (III) reducing the power of a compound is related to its ability to transfer electrons and serves as a useful indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant reaction (Rohman *et al*., 2010). The presence of antioxidants in the spice and herbal extracts causes the reduction of the $Fe^{3+/}$ferricyanide complex to the ferrous form. Therefore, the concentration of Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm (Amarowicz *et al*., 2004). A higher absorbance value indicates a higher reduction capacity. The ferric reducing power of *Rosemary*, *Tosign*, and *Koseret* ethanol extracts and ascorbic shown are shown in Figure 2. Similar to DPPH scavenging activity, the ferric reducing power increased with increasing the concentration of the extracts (Hailemariam, 2013) reported that the reductive ability of *Tosign* extract has been found that the Fe^{3+} to Fe^{2+} transformation occurred mainly in the presence of phenolic compounds in the extract. In addition, Engeda *et al*. (2015) reported that the methanolic extract of *Tosign* had the strongest ferric reducing power, which has been correlated with the amount of total phenolic and flavonoid contents.

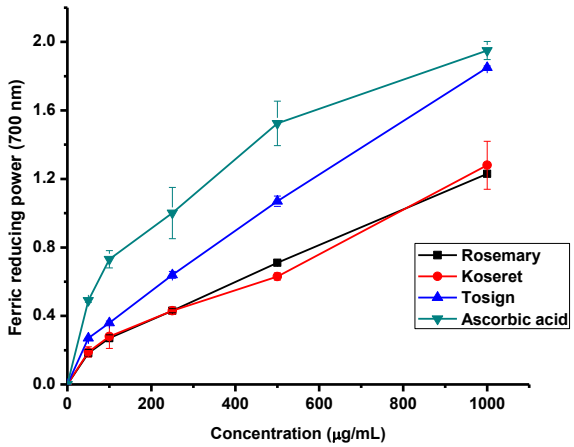

Figure 2. Ferric ion reducing power of ethanol extracts of *Rosemary*, *Tosign* and *Koseret* and the reference (ascorbic acid) at different concentrations (µg/mL). Values are the average of triplicate measurements (mean±SD).

The IC_{50} value of ferric reducing power was calculated from the absorbance against the concentration of the extract (Table 2). Similar to DPPH scavenging, *Tosign* extract had significantly ($p < 0.05$) the lowest IC_{50} value (strongest ferric reducing power) compared to *Koseret* and *Rosemary* extracts. Ascorbic acid showed a stronger ferric reducing power activity (the smallest IC_{50}) than that of *Rosemary, Tosign,* and *Koseret* extracts. No significant difference ($p > 0.05$) was observed in ferric reducing power between *Koseret* and *Rosemary* extracts, but these values showed significantly weaker reducing power ($p < 0.05$) than that of *Tosign* extract. This difference might be the amount and type of phenolic compounds present in the extracts (Ayoola *et al*., 2008; Jemal *et al*., 2011).

3.2.3 Iron chelating activity

Transition metals have been proposed to be the catalysts for the initial formation of radicals. Chelating agents may stabilize transition metals in the living systems and inhibit radical generations, consequently reducing free radical damage. Metal chelating agents may have a dramatic effect on increasing the oxidation stability by blocking the pro-oxidant metal ions, and thus limiting the formation of chain initiators by preventing metal-assisted homolysis of hydroperoxides in lipid peroxidation (Praveen *et al*., 2012).

To better estimate the potential antioxidative properties of the extracts, the chelating activity of each extract was evaluated against Fe^{2+}. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in the red colour of the complex. Measurement of the colour intensity reduction at 562 nm wavelength allows estimation of the metal chelating activity of the chelators (Yamaguchi *et al*., 2000). In this assay, both the extracts and reference control (EDTA) were assessed for their ability to compete with ferrozine for Fe^{2+} in the solution. The percentage of iron-chelating activities of all extracts and references was concentration -dependent (from 100 to 1000 µg/mL) (Figure 3).

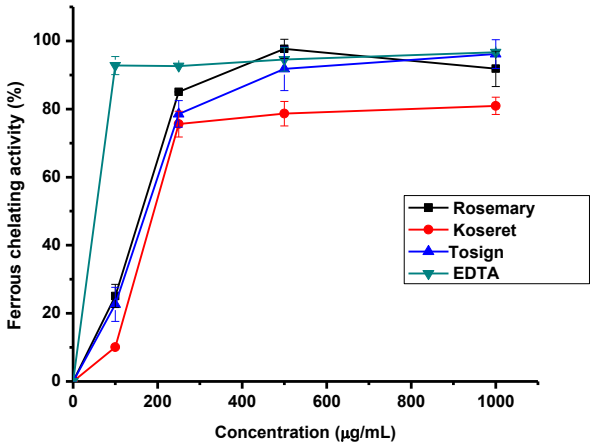

Figure 3. Iron chelating activity of *Rosemary*, *Tosign* and *Koseret* ethanol extracts and reference (EDTA, excellent chelator of ferrous ion) at different concentrations. Values are the average of triplicate measurements (mean±SD).

The IC_{50} values of ferrous ion chelating were calculated from a graph of iron chelating activity against the concentration of the extracts (Table 2). The lower the IC_{50} value, the higher the antioxidant activity. The IC_{50} values *Rosemary* and *Tosign* extract had no significant ($p > 0.05$) difference, but these values showed stronger iron -chelating scavenging activity (lower IC_{50} value) than that of the *Koseret* extracts. This could be in the presence

of more chelating agents present in *Rosemary* extract, and the complex formation is disrupted, resulting in a decrease in the red colour of the complex. EDTA was an excellent chelator for ferrous ions, and its chelating capacity (IC_{50} = 51.17±2.59 mg/mL) was much stronger than that of the ethanol extracts of *Rosemary*, *Tosign, and Koseret* ($p < 0.05$). Engida *et al*. (2015) reported methanol extract of *Tosign* collected from the central highlands of Ethiopia showed weaker iron-chelating activity than that of the present study. This may be the solvent type used and the geographical location and season of harvesting. The Rosemary extract of the present study showed stronger ferrous chelating activity than that of samples collected from different countries (Nateqi and Mirghazanfari, 2018; Oney-Montalvo, 2020).

3.3 Peroxide value

The peroxide values of *Rosemary*, *Tosign* and *Koseret* extract-treated beef samples showed lower initial peroxide values and a lower range of increase with time compared to the control (Figure 4). A sharp increase in the level of peroxide was observed in the control sample after 3 days of storage, and its rate was exponentially increasing up to day 12 storage at 4°C. It could be due to the presence of pro-oxidants which speeded the auto-oxidation process and yielded higher concentrations of oxidation product (Mohd *et al*., 2008). Among the antioxidants used, the beef samples treated with BHT (commercial synthetic antioxidant) showed lower initial peroxide values and lower increase ranges compared with other herbal additives. Compared with the control, the extract samples and reference (BHT) showed a lower increase in peroxidation levels over 15 days of testing. On day 12 of testing, the inhibition percentages of BHT, *Rosemary*, *Tosign*, *Koseret*, and control were 0.169±0.191, 0.179±0.191, 0.247±0.061, 0.280±0.236, 0.55±0.28 meq peroxide/Kg of beef, respectively. The increment of peroxide value of all treatments and control throughout the storage period of 15-day storage was due to the autocatalytic nature of the lipid oxidation reaction. In general, these results indicated that *Rosemary* extract was more effective in reducing the formation of peroxides in beef during storage at 4°C than that of *Koseret* and *Tosign* extracts.

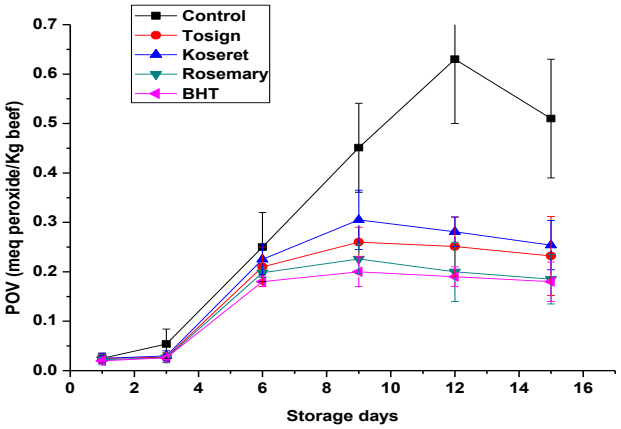

Figure 4. Changes in peroxide values of beef samples incorporated with *Rosemary, Tosign*, *Koseret* and BHT extracts during storage at 4°C. Control used is the ground raw beef in the absence of extracts. Values are the average of triplicate measurements (mean±SD).

3.4 Thiobarbituric acid reactive substances assay

During lipid peroxidation, lipid peroxides are formed, with a subsequent formation of peroxyl radicals, followed by a decomposition phase to yield the aldehydes such as hexanal, malondialdehyde, and 4-hydroxynonenal. This assay is based on the detection of a deep orange colour developed during the heating at 85°C, which is formed between the reaction of malondialdehyde and thiobarbituric acid (TBA) at a late stage of lipid oxidation in the aqueous phase.

A comparison of secondary products of lipid peroxidation measured as a percentage of inhibition of TBARS was shown in Table 3. As the day of storage increased, the inhibition of the formation of malondialdehyde in control samples decreased, and the lowest inhibition was observed on day 15. Similarly, the inhibition potential of all samples and BHT decreased with increasing days of storage. Beef treated with the *Rosemary* extract was more effective than that of *Koseret* and *Tosign* extracts in reducing lipid oxidation by

Table 3. Percentage of inhibition of thiobarbituric acid reactive substances (TBARS) of *Rosemary*, *Tosign* and *Koseret* extracts incorporated in the beef sample during the storage at 4°C.

Storage days	Control	*Tosign*	*Koseret*	*Rosemary*	BHT
1	-	-	-	-	-
3	$93.24±3.00^{aE}$	$94.96±4.50^{aCD}$	$95.65±5.22^{aC}$	$95.04±5.3^{aBC}$	$96.33±4.80^{aC}$
6	$81.85±2.20^{aD}$	$92.20±3.92^{bC}$	$90.30±4.11^{bC}$	$93.56±3.1^{bB}$	$93.90±3.85^{bBC}$
9	$76.11±4.21^{aC}$	$89.91±3.46^{bC}$	$87.25±6.02^{bB}$	$90.55±3.4^{bB}$	$91.56±4.60^{bB}$
12	$64.87±3.38^{aB}$	$82.65±5.93^{bB}$	$82.41±3.60^{bB}$	$84.63±4.4^{bA}$	$87.40±6.61^{cA}$
15	$46.65±1.90^{aA}$	$75.33±3.20^{bA}$	$69.95±4.34^{bA}$	$80.20±2.3^{cA}$	$83.81±5.50^{cA}$

Values are presented as mean±SD (n = 3). Values with different lowercase superscript within the same column and values with different uppercase superscript within the same row are significantly different at $p < 0.05$. BHT: Commercial synthetic antioxidant.

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lowering the level of malondialdehyde value. At the end of storage the percentage of inhibition of TBARS was decreased in the order BHT (83.81±5.50%) > *Rosemary* (80.20±2.3%) > *Tosign* (75.33±3.20%) > *Koseret* > control (46.65±1.90%) respectively. Therefore, the antioxidant activity of *Rosemary* extract is considered to be stronger in the inhibition of lipid oxidation than the rest extracts. *Tosign* and *Koseret* extracts showed weaker antioxidant activity ($p < 0.05$) than that of BHT, while the *Rosemary* extract showed similar activity with BHT ($p < 0.05$). This might be because phenolic compounds are considered to be one of the quick inhibitors of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids (Jayathilakan *et al*., 2007).

4. Conclusion

According to the results of this study, the antioxidant activities of the tested extracts were closely associated with their total phenolic contents. Based on measured results, it might be concluded that the addition of *Koseret*, *Tosign*, and *Rosemary* extracts exhibited inhibitory potential on lipid oxidation of beef meat during storage. However, further studies are needed to evaluate the potential of various extracting solvents, a mixture of solvents and individual bioactive compounds. Furthermore, the research revealed the bioactive compounds present in the crude extracts of these dietary herbs have the potential to be used as possible natural substitutes for controversial synthetic antioxidants currently used in food preservation.

Conflict of interest

The authors declare no conflict of interest.

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