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RESEARCH

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Evaluation of seasonal variation, effect of extraction solvent on phytochemicals and antioxidant activity on *Rosmarinus officinalis* grown in different agro-ecological zones of Kiambu County, Kenya

Zelipha N. Kabubii[1,2*], James M. Mbaria[1], Mbaabu P. Mathiu[3], John M. Wanjohi[4] and Evans N. Nyaboga[2]

Abstract

Background Rosemary (*Rosmarinus officinalis*) is a commonly used culinary herb with great potential applications in the pharmaceutical, food, and cosmetics industries because of its reported bioactive phytochemicals and antioxidant properties. The purpose of the study was to investigate the effect of seasonal variations in different agro-ecological zones (AEZs) on the phytochemical content and corresponding antioxidant activities of *R. officinalis*, to ascertain the best growth period at which the plant possesses the highest phytochemical components and most potent antioxidant property. The study also aimed at comparing different extraction solvents to establish the best extraction system for the bioactive compounds.

Methods The leaves of *R. officinalis* were harvested from six purposively selected sites in four agro-ecological zones of Kiambu County, Kenya both in the wet and the dry seasons. Phytochemicals were extracted in 80% methanol, 80% ethanol, and distilled water. Total phenolic content (TPC), total flavonoids content (TFC), and total tannins content (TTC) were measured spectrophotometrically as gallic acid equivalent (GAE), rutin equivalent (RUTE) and tannic acid equivalent (TAE), respectively. The antioxidant activities were measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP). The 80% ethanolic solvent was used to compare the phytochemical content and corresponding antioxidant activities of *R. officinalis* leaf samples collected from two consecutive seasons in different agro-ecological zones.

Results The solvents showed no significant difference ($P > 0.05$) in TPC with ethanol repotting the highest followed by methanol and water ranging from 39.71 ± 6.77, 24.91 ± 5.15 and 24.91 ± 7.30 (mg/g GAE), respectively. The aqueous TFC (117.22 ± 3.64 mg/g RUTE) was the highest followed by ethanolic and methanolic with 34.72 ± 2.13 and 16.86 ± 2.80 mg/g RUTE, respectively. The TTC of water, methanol, and ethanol extracts were; 19.88 ± 4.59, 15.02 ± 1.25, and 4.27 ± 1.48 mg/g TAE, respectively. The DPPH activity between methanol and ethanol extracts showed no significant difference. The FRAP activity also showed no significant difference ($P > 0.05$) among the three solvents. There were significant differences between the wet and dry seasons in the phytochemical content. There was no recorded significant difference in the DPPH activity between the dry and wet season in all AEZs. FRAP was

*Correspondence:
Zelipha N. Kabubii
zelipha.kabubii@uonbi.ac.ke
Full list of author information is available at the end of the article

significantly higher in the dry season than the wet season for *R. officinalis* leaves harvested in all agro-agroecological zones except Thika. There were significant differences in phytochemical content and antioxidant activity between the agro-ecologicalzones ($p < 0.05$) except for the TFC.

Conclusions The data obtained from this study demonstrated that hydro-alcoholic /methanolic and aqueous maceration systems extracted bioactive compounds from *R. officinalis* with high potential for applications in industries**.** The *R. officinalis* from different agro-ecological zones contained variable phytochemical composition, suggesting that geographical location and climatic conditions influence the biosynthesis and accumulation of secondary metabolites and other bioactive compounds. The data provided in this study will be crucial for processors to select the optimal harvesting season for the extraction of desired bioactive compounds from *Rosmarinus officinalis*.

Keywords Agro-ecological zones, Antioxidant, Extraction, Flavonoids, Phenolic, Season, Tannins

Introduction

Many medicinal plants have been found to have therapeutic/protective activities against non-communicable and infectious diseases due to the presence of bioactive phytochemicals and many secondary metabolites (Rehman and Akash 2017). Flavonoids, phenolics, saponins, and tannins are among the many phytochemicals that are rich in constitutes active against various conditions such as cancer (Ochwang'i et al. 2016) and diabetes (Mbithi et al. 2018). Antioxidants are very useful against oxidative stress caused by the formation of reactive oxygen species such as superoxide (O_2^-), Hydroxyl (OH^-), and peroxyl (OOH, ROO) (Unsal et al. 2020). The pathogenesis of diseases such as diabetes, cancer, atherosclerosis, and Alzheimer's is found to be increased by the presence of the reactive oxygen, species (Chen et al. 2020)

Rosemary (*Rosmarinus officinalis* L.) is a common herb originating from the Mediterranean region and commonly used for culinary purposes. Besides seasoning, it is also used as a natural preservative in the food industry (Nieto et al. 2018). Pharmacological studies have demonstrated that *R. officinalis* has bioactivity against various conditions such as asthma (Sinkovic et al. 2011), renal colic (Gonçalves et al. 2019), peptic ulcer, inflammation (Borges et al. 2019), hyperglycemia (Hegazy et al. 2018), cancer (Nassazi et al. 2020), microbial and antiviral activity (de Oliveira et al. 2019). These medicinal applications are due to a wide range of phytoconstituents in *R. officinalis* including flavonoids, alkaloids, tannins, saponins, camphor, caffeic acid, ursolic acid, betulinic acid, rosmarinic acid and carnasoic acid (de Macedo 2020; Andrade et al. 2018; de Oliveira et al. 2019).

The biosynthesis of phytochemicals and other secondary metabolites in plants is mainly dependent on genetic makeup. The presence and quantities of secondary metabolites also are significantly affected by several factors including environmental stress such amount of rainfall, humidity, light exposure and ultraviolet (UV) intensity with the volatile compounds affected more severely (Usano-Alemany et al. 2014). Other factors that can cause variations include soil type, plant maturity, and nutritional content of growing areas (Nchabeleng et al. 2012; Demasi et al. 2018). According to Figueiredo et al. (2008), a variety of pathogens infecting plants in a specific season can also affect the quantities of specific secondary metabolites. Therefore, there is a need to ascertain the season at which the plant species possess the highest quantity of phytochemicals and the most potent antioxidant capacity. In addition, the choice of solvent in the extraction process dictates the phytochemical compounds and other secondary metabolites in the extract, thus affecting its functionality and properties (Dirar et al. 2019). This study aims at evaluating the effect of two consecutive seasons on phytochemicals and antioxidant activities of leaf extracts (water, 80% ethanol, and 80% methanol) of *Rosmarinus officinalis* cultivated in different agro-ecological zones in Kiambu County, Kenya. This will provide valuable information to food processing, pharmaceutical, and other industries as guidelines for the best growing season and efficient solvent for the extraction of desired bioactive compounds from *Rosmarinus officinalis*.

Materials and methods

Study area

The study was conducted in four sub-Counties (Lari, Kiambaa, Juja, and Thika) of Kiambu County, Kenya (Fig. 1). According to the Food and Agriculture Organization (FAO 1996), Kiambu County is divided into four broad agro-ecological zones (AEZs): (1) Upper Highland between 1800 and 2550 m above sea level, (2) Lower Highland between 1500 and 1800 m above sea level, (3) Upper midland between 1300 and 1500 m above sea level and (4) Lower midland zone between 1200 and 1300 m above sea level. Each of the sub-Counties represented a different agro-ecological zone (Table 1).

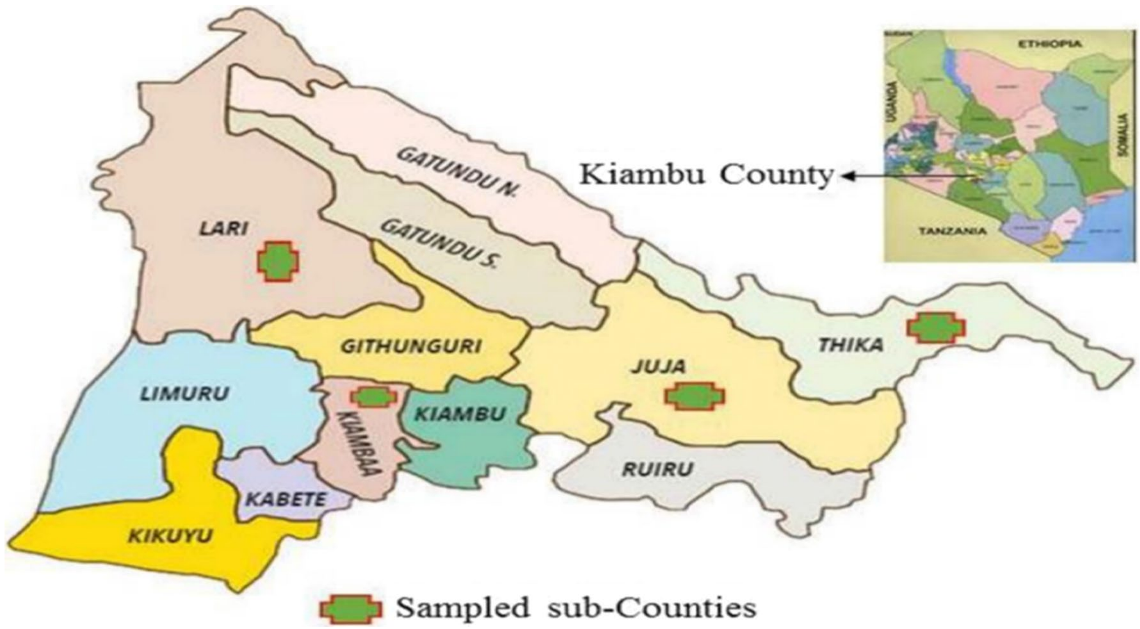

Fig. 1 Map of Kiambu County showing the location of study areas: Lari, Kiambaa, Juja, and Thika sub-Counties (Google Maps 2021)

Plant material collection and preparation of powder

The *R. officinalis* plants sampled from all the agro-ecological zones were identified in the herbarium unit, Department of Biology, University of Nairobi, and the specimens were given a number and deposited in the herbarium. The aerial parts (10 cm) of the plants were harvested from three different plants for each of the 6 sites in each agro-ecological zone for two consecutive seasons; (i) the month of April 2019 of the wet season and (ii) the month of August 2019. of the dry season, Samples were washed with clean running tap water and rinsed with distilled water after which they were air dried for 21 days in a room maintained at 25 °C to a moisture content of 12–15%. The dried leaves were then separated from the **s**talks and milled to a fine powder using an electric grinder. The powder was placed in plastic packaging bags, sealed, and stored in a refrigerator at 4 °C for subsequent use.

Preparation of crude extracts for phytochemical and antioxidant assays

Three different solvents were used namely; 80% ethanol, 80% methanol (analytical grade), and distilled water for the preparation of the crude extract. Leaf powder (2 g) was weighed into 100 ml sample bottles and 20 ml of respective solvents was added. The mixture was incubated in a shaker for 48 h at 24 °C at a moderately low speed, a slight modification of the method described by Adan et al. (2020). The content was centrifuged at 3000 revolutions per minute (rpm) for 5 min and the supernatant (crude extract) was placed in bottles, sealed, and stored under refrigeration (4 °C) awaiting the assays. The crude extracts were used within 6 h.

Quantitative phytochemical evaluation

Estimation of total phenolic compounds, flavonoids, and tannins was performed by using the UV–VIS spectrophotometer (UVmini-1240, Shimadzu Europe). All the standard chemicals including rutin, gallic acid, and tannic acid were analytic grades purchased from Sigma-Aldrich unless otherwise stated.

Determination of total phenolic content (TPC)

Total phenolic content was carried out using a method described by Ojwang et al. (2018). The methanol, ethanol, and water sample extract (0.1 ml) were mixed with 3 ml of solvent; 80% methanol, 70% ethanol, and distilled water, respectively. After mixing thoroughly, 0.5 ml of Folin Ciocalteau reagent, and 2 ml of 20% sodium carbonate were added and the content was thoroughly mixed and incubated for 20 min at 23 °C Absorbance was read at 725 nm and a gallic acid standard at the concentrations of 20, 40, 60, 80, and 100 mg/ml was used. The assays were performed in triplicates. The results of samples were expressed as mg gallic acid equivalent (GAE) per g of dry powder using the standard curve equations that were generated ($y = 0.123 + 0.0104$, $y = 0.0368x + 0.0138$ and $y = 0.057x + 0.0345$) for methanol, ethanol, and aqueous extracts, respectively.

Table 1 Agro-ecological zones for the four sub-Counties in Kenya showing the environmental conditions during the dry and wet seasons, and sampling sites for *Rosmarinus officinalis leaves*

Zone description altitude in m (above sea level)	Zone	Sampling sites	Coordinates	Parameter	Wet season Mid-March–June		Dry season July–September	
					Lowest	Highest	Lowest	Highest
Upper Highland 1800–2550	Lari	1. Kirenga 2. Gitithia 3. Gituamba 4. Nyambari 5. Githirioni 6. Rukuma	1°01′00.0"S 36°38′00.0"E	Temperature (°C)	23	28	23	25
				Humidity (%)	65	83	66	72
				Rainfall (mm)	119	269	66.5	74.5
				UV Index	5	5	4	5
				Rainfall days	3	11	3	4
				Sun hours	325.9	355.1	349.2	357
Lower Highland 1500–1800	Kiambaa	1. Ruaka 2. Ndenderu 3. Kihara 4. Muchatha 5. Gathiga 6. Karura	1°11′00.0"S 36°45′00.0"E	Temperature (C)	23	28	23	25
				Humidity (%)	65	83	66	72
				Rainfall (mm)	111.9	260	66.54	74.5
				UV Index	5	5	4	5
				Rainfall days	3	11	3	4
				Sun hours	325.9	355.8	349.2	357
Upper Midland 1300–1500	Juja	1. Murera 2. Mugutha 3. Gitothua 4. Kihunguro 5. Kalimoni 6. Juja Farm	1°11′00.0"S 37°07′00.0"E	Temperature(°C)	24	28	23	26
				Humidity (%)	66	83	66	73
				Rainfall (mm)	90.3	240	70.34	77
				UV Index	5	5	4	5
				Rainfall days	4	10	4	5
				Sun hours	332.1	354.2	351.4	358.4
Lower Midland 1200–1300	Thika	1. ThikaTown 2. Gatuanyaga 3. Makongeni 4. Munyu, 5. Ngoliba 1 6. Ngoliba 2	1.0490° S, 37.2651° E	Temperature (°C)	24	28	23	26
				Humidity (%)	66	83	66	73
				Rainfall (mm)	90.3	240	70.3	77
				UV Index	5	5	5	5
				Rainfall days	4	10	3	4
				Sun hours	325.5	354.2	349.5	358.4

Source: World weather online (2021) and Kenya Meteorological Department (2019)

Determination of total flavonoid content (TFC)

Total flavonoid content was based on the formation of the aluminum complex as per the method described by Kimondo et al. (2019). The corresponding extract (100 µl) was added to 1 ml (2%) aluminum chloride and 2 drops of acetic acid were added. The mixture was made to 5 ml with respective solvents and incubated for 30 min at 23 °C after which absorbance was read at 415 nm. The tests were performed in triplicates. A standard curve of rutin was constructed using 20, 40, 60, 80, and 100 mg/ml rutin. TFC was expressed as mg rutin equivalent per g sample using the equations obtained: $y = 0.0163x + 0.0565$, $y = 0.0168x + 0.0079$, and $y = 0.0023x + 0.0313$ for methanol, ethanol and aqueous solvents, respectively.

Determination of total tannin content (TTC)

The total tannins present in the extracts were analyzed using the procedure described by Adan et al. (2020). The extracts (0.1 ml) were added to 3 ml of their respective solvents and 2 ml of 20% sodium carbonate was added. Then the mixture was stirred and allowed to stand for 5 min, after which 0.5 ml of the Folin Ciacateu reagent was added. The mixture was incubated at room temperature for 20 min and absorbance read at 700 nm. The measurements were performed in triplicates. The tannic acid standard curve was constructed using 20, 40, 60, 80, and 100 mg/ml. The total tannin was calculated from the equations obtained: $y = 0.157x + 0.323$, $y = 0.0065x + 0.0367$, and $y = 0.055x + 0.312$ for solvents methanol, ethanol, and aqueous, respectively. The results were expressed as mg tannic acid equivalent per gram dry sample.

In-vitro antioxidant activity assays

The antioxidant capacity of the crude extracts of *R. officinalis* was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The DPPH scavenging activity of the crude extracts was determined colorimetrically according to the method described by Adan et al. (2020). A total of 2 ml of methanol solution of 0.1 mM DPPH solution was mixed with 0.1 ml sample solution and incubated in the dark for 30 min and absorbance read at 517 nm. In a control experiment, 2 ml of methanol and 0.1 ml of the respective solvents were used. All the experiments were performed in triplicates. The DPPH free radical scavenging activity was expressed as a percentage using the formula: $(A_{control} - A_{sample}/A_{control}) \times 100$, where $A_{control}$ and A_{sample} denote the absorbance of the control and the sample, respectively.

Ferric-reducing antioxidant power (FRAP)

A modification of the method described by Sylvie et al. (2014) was used to estimate the total reducing power of the extracts prepared using three different solvents. An aliquot of 0.1 ml of each extract was transferred into a test and mixed with 1 ml of 0.2 mM phosphate buffer (pH 6.6) and 1 ml of potassium ferricyanide (1%). The mixture was incubated in a water bath at 50 °C for 20 min before the addition of 1 ml of 10% trichloroacetic acid and then centrifuged at 3000 rpm for 10 min. The upper phase (2 ml) of the solution was mixed with an equal volume of distilled water and 0.1 ml of 0.1% ferric chloride ($FeCl_3$). The absorbance of the mixture and control was read at 593 nm. The controls contained all the test reagents except the crude extract. All the measurements were performed in triplicates.

Statistical analysis

The raw data was handled in Microsoft excel 2010 for computation of mean and standard deviation. One-way ANOVA was used to examine whether there was any significant difference in phytochemicals and antioxidant activity levels among the three solvents with 5% levels of significance. The same was also applied to examine the significant difference in the various agro-ecological zones in two consecutive seasons (dry and wet) using the statistical package for social sciences (SPSS) version 26 software.

Results

The influence of solvent on TPC, TFC, TTC, and antioxidant activity

The effect of different solvents (80% methanol, 80% ethanol, and aqueous) on total phenolics, flavonoid, and tannins contents are presented in Table 2. Ethanol extract showed the highest TPC followed by methanol and water with 39.71 ± 6.77 (mg/g GAE), 24.91 ± 5.15 (mg/g GAE), and 24.91 ± 7.30 (mg/g GAE), respectively. However, there was no significance of the TPC between the three solvents used. Aqueous extract gave the highest TFC (117.22 ± 12.64 mg/g RUTE) that was significantly higher, ($p < 0.05$) compared to ethanol (34.72 ± 6.51 mg/g RUTE) and methanol (16.86 ± 2.80 mg/g RUTE). The TTC was highest in the aqueous extract (19.88 ± 4.59 mg/g TAE) followed by ethanol (15.02 ± 1.25 mg/g TAE). with significantly lower content in methanol (4.27 ± 1.48 mg/g TAE). The DPPH methanol extract (82.35 ± 12.14%) was the highest followed by ethanol (80.00 ± 12.1%) and significantly low activity in water (33.03 ± 4.69%). The FRAP was highest in water extracts (0.84 ± 0.05) but was not significantly different from methanol.

Effect of agro-ecological zones on TPC, TFC, TTC, and antioxidant activity

The 80% ethanol extract was used to evaluate the TPC, TFC, TTC, and antioxidant activity and the results showed in Table 3. The TPC varied from 14.91 ± 1.17 (Lari) to 57.47 ± 8.21 mg/g GAE (Kiambaa). Total

Table 2 Effect of solvent extraction method on the total phenolic content, total flavonoid content, total tannin content, percent of 2,2-diphenyl-1-picrylhydrazyl inhibition and ferric reducing antioxidant power activity absorbance of *Rosmarinus officinalis* leaves

Extracting solvent	TPC (mg/g GAE)	TFC (mg/g RUTE)	TTC (mg/g TAE)	DPPH inhibition (%)	FRAP activity (absorbance)
80% ethanol	39.71 ± 6.77 ns	34.72 ± 6.51 c	15.02 ± 1. 25 a	80.00 ± 12.14 a	0.58 ± 0.4 a
80% methanol	24.91 ± 5.15 ns	16.86 ± 2.80 a	4.27 ± 1.48 b	82.35 ± 12.1 a	0.74 ± 0.11 ab
Aqueous	24.91 ± 7.30 ns	117.22 ± 12.64 b	19.88 ± 4.59 a	33.03 ± 4.69 b	0.84 ± 0.05 b
p-value	0.219	0.0001	0.0002	0.0001	0.339

Results are means from leaves harvested from four agro-ecological zones in Kenya. Values bearing the same letter in the column are not statistically different ($P < 0.05$). Values are expressed as mean ± Standard Deviation

TPC total phenolic content, *TFC* total flavonoids content, *TTC* total tannins content, *DPPH* diphenyl-1-picrylhydrazyl inhibition, *FRAP* ferric reducing antioxidant power, *GAE* gallic acid equivalent, *RUTE* rutin equivalent, *TAE* tannic acid equivalent, *ns* no significant differences

Table 3 Effect of agro-ecological zones on the total phenolic content, total flavonoid content, total tannin content, percent of 2,2-diphenyl-1-picrylhydrazyl inhibition and ferric reducing antioxidant power activity absorbance of *Rosmarinus officinalis* leaves

Agro-ecological zones	TPC (mg/g GAE)	TFC (mg/g RUTE)	TTC (mg/g TAE)	DPPH inhibition (%)	FRAP activity (absorbance)
Lari	14.91 ± 1.17 [a]	42.16 ± 4.03 [ns]	30.4 ± 2.53 [a]	92.47 ± 2.92 [a]	0.88 ± 0.08 [a]
Kiambaa	57.47 ± 8.21 [c]	32.77 ± 3.51 [ns]	8.51 ± 1.10 [c]	81.60 ± 2.50 [b]	0.68 ± 0.04 [b]
Juja	24.52 ± 1.54 [b]	29.69 ± 8.42 [ns]	19.19 ± 1.14 [b]	61.23 ± 5.71 [c]	0.51 ± 0.06 [c]
Thika	53.52 ± 7.86 [c]	34.20 ± 2.13	4.95 ± 1.99 [c]	84.70 ± 1.43 [b]	0.27 ± 0.03 [d]
p-value	0.0001	0.476	0.0003	0.0002	0.0001

Results are means from three different extraction methods. Values bearing the same letter in the column are not statistically different ($P < 0.05$). Values are expressed as mean ± Standard Deviation

TPC total phenolic content, *TFC* total flavonoids content, *TTC* total tannins content, *DPPH* diphenyl-1-picrylhydrazyl inhibition, *FRAP* ferric reducing antioxidant power, *GAE* gallic acid equivalent, *RUTE* rutin equivalent, *TAE* tannic acid equivalent

phenolic content between the agro-ecological zones showed significant differences except between Kiambaa and Thika ($p = 0.06$; Table 3. The mean TFC showed no significantly difference ($p > 0.05$) with the highest in Lari and lowest Juja AEZ with 42 ± 4.03 and 29.69 ± 8.42 mg/g RUTE, respectively. Highest TTC was recorded in Lari with 30.4 ± 2.53 mg/g TAE with the lowest from Thika (4.95 ± 1.99 mg/g TAE). The DPPH scavenging activity was highest in Lari (92.47 ± 2.92%) and lowest in Juja (61.23 ± 5.71%). The FRAP was highest in Kiambaa and lowest in Thika AEZs with an absorbance of 1.03 ± 0.03 and 0.74 ± 0.11, respectively. There were significant differences in TTC, DPPH, and FRAP activity between one zone and the other as shown in Table 3.

Effect of seasonal dynamics on TPC, TFC, and TTC

The effect of seasonal dynamics on the content of phytochemicals in 80% ethanol extracts was determined in leaves of *R. officinalis* (Table 4). TPC in all 4 agro-ecological zones showed significant differences ($p < 0.05$) between the wet and dry seasons. Lari and Thika had significantly higher TPC in the dry season than in the wet season while Kiambaa and Juja reported higher yields in the wet season than in the dry season. Lari, Kiambaa, and Juja AEZs recorded significantly higher levels of TFC in the leaves collected in the wet season than in the dry season. The total tannins content was higher in the dry season than in the wet seasons in all 4 agro-ecological zones (Table 4).

Table 4 The effect of seasonal dynamics (dry and wet seasons) on the total phenolic content, total flavonoid content and total tannin content of *Rosmarinus officinalis* leaves harvested in four agro-ecological zones in Kenya

Phytochemical compound	Agro-ecological zones	Dry season	Wet season	p-value
Total phenolic content (mg/g GAE)	Lari	18.11 ± 1.22	11.71 ± 1.12	0.001
	Kiambaa	49.92 ± 2.21	71.85 ± 7.87	0.010
	Juja	15.30 ± 1.15	37.73 ± 1.93	0.038
	Thika	67.45 ± 0.01	45.59 ± 2.00	0.0001
	Mean	37.70 ± 11.19	41.72 ± 3.63	0.528
Total flavonoid content (mg/g RUTE)	Lari	38.42 ± 3.66	45.89 ± 4.4	0.049
	Kiambaa	28.54 ± 4.03	37.00 ± 3.0	0.016
	Juja	11.07 ± 2.80	48.31 ± 2.04	0.001
	Thika	35.27 ± 1.08	33.12 ± 3.17	0.363
	Mean	28.325 ± 2.89	41.78 ± 2.89	0.002
Total tannin content (mg/g TAE)	Lari	34.61 ± 2.55	20.76 ± 1.22	0.002
	Kiambaa	12.50 ± 3.03	4.96 ± 0.95	0.006
	Juja	21.77 ± 11.52	16.60 ± 0.76	0.610
	Thika	6.98 ± 1.09	3.96 ± 0.89	0.036
	Mean	18.88 ± 4.55	11.57 ± 1.01	0.087

Results are means from the ethanolic extraction method. Values are expressed as mean ± Standard Deviation

TPC total phenolic content, *TFC* total flavonoids content, *TTC* total tannins content, *GAE* gallic acid equivalent, *RUTE* rutin equivalent, *TAE* tannic acid equivalent

Effect of seasonal dynamics on antioxidant activities

The effect of seasonal dynamics on the antioxidant activities in 80% ethanol extracts of *R. officinalis* leaves was determined based on DPPH and FRAP assays as shown in Table 5. The DPPH activity showed no significant difference ($p > 0.05$) between the dry and wet seasons. However, higher DPPH radical scavenging activity was recorded in the dry season in three of the agro-ecological zones. FRAP activity was higher in the dry.

Discussion

Plant natural products and their derivatives are important aspects of drug discovery (Newman and Cragg 2016; Cheuka 2016; Sorokina 2020, Gakuya et al. 2020) and by the year 2050 the herbal remedies market economy is estimated to grow by 50% (WHO 2019). Therefore, in addition to identifying medicinal plants rich in natural antioxidants and other bioactive components, it is important to determine the best time to harvest plants that have the maximum amount of the preferred compounds. The present study evaluated the contents of some phytochemical and antioxidant activities of *R. officinalis* leaves in the dry and wet seasons from different agro-ecological zones. In plant-environment interactions play a fundamental role in the biosynthesis of secondary metabolites. The content of phytochemicals and antioxidant activities can change according to the developmental and phonological stages of the plants and the environmental conditions. In *R. officinalis*, the results showed significant effects of the season of leaves collection on the variation of phytochemical content and antioxidant activities from one zone to another. This could help define the proper season to maximize the required components in *R. officinalis* leaves in the particular zone.

The bioactivity of herbal extracts depends on the chemical profile. The choice of solvent is important in the extraction process as it directly affects the chemical composition of the extracts and the extraction yield. Among the solvents available for plant extraction, hydro-alcoholic mixtures have been reported to extract a wide range of phytochemical compounds (Jacotet-Navarro et al. 2018). In *R. officinalis*, a hydro-alcoholic mixture with 50–80% ethanol has been reported to give the highest extraction yields and total phytochemical compounds in comparison to lower ethanol levels. In this study, a hydro-alcoholic mixture with 80% ethanol yielded the highest TPC in agreement with Jacotet-Navarro et al. (2018). Flavonoids are more polar in nature (Chaves et al. 2020) and this explains why the extraction of flavonoids with water yielded the highest content. Continuous shaking in maceration extraction helps in breaking the surface tension and therefore increasing the solvent penetration into the matrix of the particles. Comparative high TFC from aqueous solvent was reported by Li et al. (2017). This study showed that either water or methanol solvents could be well utilized for tannin extraction since the TTC yield had no significant difference between these two solvents. Optimization of certain parameters such as extraction time, sonication, and temperatures has been reported to show significant differences in yields (De Hoyos-Martínez et al. 2019). The high DPPH activity of the methanol and ethanol extracts in comparison with water demonstrates that a high-polarity solvent is less effective for extracting antioxidants with efficient free radical scavenging activity (Adan et al. 2020). On the other hand, water was found to possess good reducing potential as indicated in the study by Li et al. (2017).

Phenolic compounds are a major source of the antioxidant properties of plants (Pereira et al. 2009). Although

Table 5 The effect of seasonal dynamics (dry and wet seasons) on the antioxidant activities of *Rosmarinus officinalis* leaves harvested in four agro-ecological zones in Kenya

Antioxidant activity	Agro-ecological zones	Dry season	Wet season	p-values
DPPH inhibition (%)	Lari	93.53 ± 1.71	91.41 ± 4.13	0.496
	Kiambaa	83.34 ± 1.73	79.85 ± 3.27	0.060
	Juja	61.83 ± 6.68	60.63 ± 4.73	0.637
	Thika	84.57 ± 1.79	88.82 ± 1.07	0.058
	Mean	80.82 ± 3.39	80.18 ± 3.31	0.92
Ferric reducing antioxidant potential (FRAP) (absorbance)	Lari	1.00 ± 0.05	0.76 ± 0.04	0.002
	Kiambaa	0.44 ± 0.03	0.924 ± 0.05	0.0001
	Juja	0.67 ± 0.06	0.34 ± 0.05	0.014
	Thika	0.30 ± 0.02	0.23 ± 0.03	0.080
	Mean	0.602 ± 0.04	0.569 ± 0.043	0.664

Results are means from the ethanolic extraction method. Values are expressed as mean ± Standard Deviation

DPPH diphenyl-1-picrylhydrazyl inhibition, *FRAP* ferric reducing antioxidant power the production of these compounds in plant species is under the control of genetic factors and pathways, they are also significantly affected by the growing or environmental conditions (Zargoosh et al. 2019). Studies have shown that seasonal variations in rainfall, temperatures, photoperiod, and light intensity can alter the levels of the polyphenolic compounds in a plant (Rezende et al. 2015). The total phenolic content in this study showed a significant difference between seasons and also between some agro-ecological zones. This could be due to several factors such as soil type, light intensity, and humidity in the different seasons and agro-ecological zones (Cirak and Radusiene 2019). TPC was highest in Kiambaa and Thika and significantly higher in the dry season in Lari and Thika than in the wet season and vice versa for Kiambaa and Juja. This is an important aspect to maximize the harvest of plant materials during the dry season for food, pharmaceutical, and other industries utilizing phenolic compounds.

Flavonoids are an important class of plant pigments and are frequently components of the human diet. They are known to possess biological properties (antioxidant, antibacterial, antidiabetic, anti-tumoral, and anti-inflammatory) due to their ability to scavenge free radicals (Brodowska 2017). *R. officinalis* is used as a traditional medicine component and studies have suggested that the flavonoids in it contribute to the reported bioactivity (de Macedo 2020; Andrade et al. 2018; de Oliveira et al. 2019). Earlier studies showed that *R. officinalis* was found to contain 24 flavonoids with the more abundant ones being flavanols and flavanones (Mena et al. 2016). The study showed that ecological differences such as altitude and weather patterns may significantly affect flavonoid content. Lari (Upper Highland zone, 2550–1800 m above sea level) showed significantly high TFC compared with the other agro-ecological zones, making it the most suitable areas to grow *R. officinalis* as a source of flavonoids. The wet season gave significantly higher TFC in 3 out of 4 zones and non-seasonal significance in 1 zone indicating that varying climatic environments can alter the content of the bioactive flavonoid contents.

Tannins from plants are large groups of phenolic structured compounds and are normally divided into three groups; hydrolyzable, condensed, and phlorotannins. Tannins have found diverse use in many types of industries such as leather tanning, animal feeds, and food industries. According to Singh and Kumar (2019), the market demand for tannins is increasing and a 5.8% growth is expected from 2016 to 2025. Tannins are also potential medicinal agents whose tannins are in plant tissues.

Pharmacological benefits include antiseptics, anticarcinogenic, and anti-inflammatory making them useful as pharmaceuticals and nutraceuticals (Singh and Kumar 2019). The dry season yielded high tannins content than the wet season in all the agro-ecological zones and significantly higher in three of the four zones suggesting that the higher temperatures, lower humidity, lower rainfall, more sunny hours, and higher UV index are favorable for the synthesis and accumulation of antioxidants inhibit free radicals using different reaction mechanisms, which include singlet oxygen quenching in the presence of cofactors (metals chelators), hydrogen donation, reduction of hydrogen peroxide, interception of lipid peroxyl radical, etc. Due to the differences in the mode of action of antioxidants, the antioxidant potential of *R. officinalis* crude extracts was evaluated using DPPH and FRAP assays. The DPPH scavenging activity did not have any significant difference between the seasons and agrees with a study by Siatka et al. (2010). Verma et al. (2015) reported differences in total phenolic compounds, flavonoids, and tannins across the seasons and zones, in agreement with our study, suggesting that geographical location and other environmental factors can contribute to variations in phytochemical and other bioactive compounds. On the other hand, the FRAP activity showed significantly higher activity in the dry season than the wet one which was in agreement with Ribeiro et al. (2020). The study has demonstrated that *R. officinalis* with potent antioxidant activity growing in the studied agro-ecological zones can be harvested either in the dry or wet season.

Conclusions

The data obtained from this study demonstrated that the leaf extracts from *R. officinalis* possess considerable amounts of phytochemical compounds and antioxidant properties. This study also shows that *R. officinalis* from different agro-ecological zones contained variable phytochemical composition and antioxidant activity, suggesting that geographical location and environmental conditions may influence biosynthesis and accumulation of secondary metabolites and other bioactive compounds. The levels of TPC were significantly higher during the dry season in Lari and Thika zones while in Kiambaa and Juja the levels were higher during the wet season. For Lari, Kiambaa, and Juja agro-ecological zones, the best season to harvest *R. officinalis* leaves is during the wet season. For the Thika zone, both dry and wet seasons would be adequate for harvesting *R. officinalis* leaves for flavonoid extraction. *R. officinalis* leaves with higher FRAP would be best harvested in the dry season in Lari, Kiambaa, and Thika while either of the seasons would be best for Juja agro-ecological zone. *R. officinalis* with high scavenging antioxidant activity could be harvested in either the dry or wet season for all the zones under study. The present study recommends genetic identification and characterization of the *R. officinalis* cultivars grown in the different agro-ecological zones of Kiambu County, Kenya. Further systematic study on the monthly fluctuation of phytochemicals, especially important bioactive compounds in different agro-ecological zones should be carried out to get a clear insight into major environmental factors and signaling compounds affecting their synthesis and accumulation.

Abbreviations

ABS	Absorbance
DPPH	2,2-Diphenyl--picrylhydrazyl
FAO	Food and Agriculture Organization
FRAP	Ferric reducing antioxidant power
RUTE	Rutin equivalent
SPSS	Statistical Package for Social Sciences
TAE	Tannic acid equivalent
TFC	Total flavonoid content
TPC	Total phenolic content
TTC	Total tannin content
WHO	World Health Organization

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Author contributions
ZNK, JMM, MMP, JMW and ENN conceptualized and designed the study. ZNK performed the experiments, analyzed and interpreted data of the experiments. ENN assisted in data analysis and interpretation. JMM, MMP, JMW and ENN supervised the research work. ZNK drafted the manuscript, which was revised by JMM, MMP, JMW and ENN. All authors read and approved the final manuscript.

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Competing interests
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Author details
[1]Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053, Nairobi, Kenya. [2]Department of Biochemistry, Faculty of Science and Technology, University of Nairobi, P.O. Box 30197, Nairobi, Kenya. [3]Department of Veterinary Anatomy and Physiology, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053, Nairobi, Kenya. [4]Department of Chemistry, Faculty of Science and Technology, University of Nairobi, P.O. Box 30197, Nairobi, Kenya.

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