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Antioxidant Potential of Eight selected Kenyan Medicinal plants

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Abstract

Adding antioxidants to our daily food may reduce the risk of diseases caused by free radicals due to oxidative stress in the human body. In this study, eight selected Kenyan medicinal plants (Terminalia brownii, Aloe secundiflora, Prunus africana, Carissa edulis, Tithonia diversifolia, Warburgia ugandensis, Croton megalocarpus and Launaea cornuta) were studied for their potential antioxidant activity. The levels of polyphenols and flavonoids were determined using standard methods. Plant parts were extracted using water and different organic solvents. Prunus africana acetone extract had the highest levels of phenolic compounds, while Tithonia diversifolia water extract had the lowest amount. The antioxidant activity was highest in Terminalia brownii acetone extract and lowest in Croton megalocarpus diethyl ether leaves extract. This trend was followed by antioxidant potency composite index of the medicinal plant extracts. Terminalia brownii extracts had the highest antioxidant activity and potential, followed by Prunus africana extracts due to the higher total phenolic content found in the latter medicinal plants. Total phenolic content was positively correlated with FRAP, ABTS and DPPH values. Based on the antioxidant potency composite index calculated and the overall antioxidant index, it can be concluded that the extracts from T. brownii bark and P. africana bark can be recommended as a potential natural source of antioxidants appropriate for utilization in nutritional and pharmaceutical fields. Further evaluation of the bioactive compounds and antioxidant activities in living models needs to be investigated.

Keywords: Antioxidant activity; DPPH; ABTS; FRAP; free radicals; polyphenols; flavonoids.

1. Introduction

The nutraceuticals, such as phenols, reduce the risk of chronic diseases by scavenging reactive oxygen and nitrogen species in the human body and are thus referred to as antioxidants [1]. Chemical reactions that occur in any living system could produce free radical species, e.g., superoxide, hydroxyl, peroxyl, and alkoxy radicals. The most important free radicals produced during metabolic reactions are radicals derived from oxygen, reactive oxygen species (ROS) [2]. When the concentration of ROS at low to moderate, it plays essential roles in cell physiology, such as cell growth regulation, pathways of cellular signal transduction, and pathogens defence [3], [4]. Overproduction of ROS are due to environmental stress like temperature, drought, pollution, excessive light intensities, and nutritional limitation [5]–[7]. Inadequacy of antioxidant mechanisms with ROS overproduction results in oxidative stress [8], [9]. This may cause protein oxidation and lipid peroxidation, leading to DNA damage in living cells [7]. Some human diseases, such as cardiovascular, atherosclerosis, inflammation, diabetes, asthma, pulmonary hypertension, retinopathy, carcinogenesis, and neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, are thought to be caused by the highly reactive potential of ROS [10], [11].

The critical concept for healthy biological systems is the balance between antioxidant defence system and ROS formation [12], [13]. Antioxidants have been proved to reduce the harmful effect of oxidative stress and improve immune function [14], [15].

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In food industry, synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), etc., were used to eliminate the harmful effect of free radicals., However, Food and Drug Administration (FDA) limited the use of synthetic antioxidants due to toxicity and carcinogenic effect [16], [17]. Therefore, there is a need to find effective antioxidants from natural sources. During the last few decades, many reports indicate that pure natural compounds and several crude extracts have antioxidant and radical scavenging activities [18]–[20]. Phenolic compounds such as phenolic acids and flavonoids have been reported to have antioxidant activity [21]–[23].

Kenya has one of the most diverse floras in East Africa, with over 7000 plant species [24]. As a result of the increased number of plant species, several medicinal plants have been discovered in the region [25]. Kenyan medicinal plants contain bioactive compounds and have been used for thousands of years to treat some health disorders and prevent different diseases in Kenya. More than 400 plant species are used to manage common diseases in East Africa documented in several ethnobotanical [26]. Besides, they are very effective when added to Kenyan daily food system [27]. This study aimed to determine eight Kenyan medicinal plants' extracts for their content of phenolic and flavonoids and the relationship between phenolic contents and antioxidant activity.

2. Materials and methods

2.1. Plant collection

Ten (10) kg of the fresh parts (leaves, roots, stem, and bark) of plant samples *Terminalia brownii, Aloe secundiflora, Prunus Africana,* and *Carissa edulis* were collected from Meka, Mbeere, Eastern region of Kenya., and *Tithonia diversifolia, Warburgia ugandensis, Croton megalocarpus, Launaea cornuta* were collected from Jomo Kenyatta university for agriculture and Technology (JKUAT) compound between June and August 2017, (shown in Table 1).

Table (1) plant name and part investigated.

Plant name	Family	Local name	Part investigated	Abbreviation
Terminalia Brownii	Combretaceae	(mwalambe,mbarao	Bark	TBB
Aloe Secundiflora	Asphodelaceae	Kil(/r)uma	Leaves, Root	ASL, ASR
Prunus Africana	Rosacceae	Red stinkwood or muiri	Bark	PAF
Carissa Edulis	Apocynaceae	Mtandamboo	Root	CDR
Tithonia Diversifolia	Asteraceae	Maua amalulu	Leaves, Stem, Root	TDL, TDS, TDR
Warburgia Ugandensis	Canellaceae	Uganda green heart tree	Leaves, Bark	WUL, WUB
Croton Megalocarpus	Euphorbiaceae	Mukinduri, msenefu	Leaves, Bark	CML, CMB
Launaea Cornuta	Asteraceae	Bitter lettuce	Whole plant	LC

The plants were identified using scientific literature and authenticated by a botanist in JKUAT. Voucher specimens were stored in the Botany department.

2.2. Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS·+), (±)-6-hydroxy-2,5,7,8-tetramethylchromone-2-carboxylic acid (Trolox), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), dimethyl sulfoxide (DMSO) Ascorbic acid, tannic acid, and quercetin were obtained from Sigma–Aldrich, Germany; Methanol, Ethanol, Ethyl acetate, Acetone, were acquired from Scharlab (Barcelona, Spain), Dichloromethane, Chloroform, Diethyl ether, Hexane, Potassium persulfate Anhydrous sodium carbonate, Ferric chloride hexahydrate ($FeCl_3.6H_2O$), Folin–Ciocalteu reagent, Hydrochloric acid, Sulfuric acid, Ammonia, Glacial acetic acid, Sodium hydroxide were purchased from LOBA CHEMIE, Mumbai, India. All chemicals used were of analytical grade.

2.3. Extraction

According to standard protocols [28], the extraction was done with some modifications. The plant materials were air-dried in shadow (temperature not exceeding 40°C and away from direct sunlight) for 3 to 4 weeks. The Air-dried sample was pre-crushed and later pulverized into a fine powder using an electric blender. The powder was stored at -18 °C in an airtight container. Briefly, 100g of each powdered sample was soaked separately in 500 ml of each solvent (water, or methanol, or ethanol, or ethyl acetate, or acetone, or dichloromethane, or chloroform, or diethyl ether, or hexane) for 72 h at room temperature with intermittent shaking. Filtration was done using Whatman No.1 filter paper. For each extract (except for water), the filtrate was concentrated under reduced pressure on a rotary evaporator (temperature of 50 to 55°C; 10 to 15 rpm). The water extract was filtered using a Buchner funnel and Whatman No. 1 filter paper. The filtrates were quickly frozen at -40°C and dried for 48 h using a freeze dryer. Then, the extracts were stored at 4°C until further analysis.

2.4. Determination of total phenolic compounds.

The total content of phenolic compounds was determined using the Folin–Ciocalteu micro method [29]. Tannic acid was used as a standard for the calibration curve. The content of total phenolic compounds was expressed as mg tannic acid equivalent (mg TAN/g of extract). All samples were analyzed in three replicates.

2.5. Determination of total flavonoids

The total flavonoid content was determined using the aluminium chloride colourimetric method [30]. For the calibration curve, quercetin was employed as a standard (5, 10, 50 and 100 µg/ml). The content of total flavonoids was expressed as mg quercetin equivalent (mg QU/g of extract). All samples were analyzed in three replicates.

2.6. Assessment of antioxidant activity

2.6.1. Antioxidant activity using DPPH Assay

The assay was determined as described by [31], [32]. Appropriate blanks (DMSO 2%) and standards (TROLOX and ascorbic acid solutions in DMSO 5, 10, 50 and 100 µg/ml) were run simultaneously. Several concentrations of extracts (10, 30, 100, 300 and 1000 µg/ml) were prepared. The percentage inhibition of free radical DPPH was calculated from the equation below according to [33].

$$\text{The \% inhibition} = \frac{\text{A blank} - \text{A sample}}{\text{A blank}} \times 100$$

where A blank is the absorbance of DPPH solution without extract at 490 nm, and A sample is the absorbance of the sample with DPPH solution.

The half-maximal inhibitory concentration (IC50) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%. All tests were measured in triplicate.

2.6.2. ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) assay

The ABTS+ technique was used to determine the antioxidant activity and capacity [34], [35]. A series of standard TROLOX (5–200 µg /ml) were prepared. The % inhibition of both standard and samples were calculated and drawn as a function of the concentration of standard. The inhibition percentage of ABTS was calculated using the following equation:

$$\text{ABTS cation radical scavenging activity (\%)} = \frac{\text{A blank} - \text{A sample}}{\text{A blank}} \times 100$$

Where A blank is the absorbance without extracts at 734 nm, and A sample is the absorbance of the test solution. The half-maximal inhibitory concentration (IC50) was reported as the amount of antioxidant required to decrease the initial ABTS concentration by 50%. All tests were measured in triplicate, and graphs were plotted using the average of three determinations.

2.6.3. Ferric reducing antioxidant power (FRAP) assay

According to [36], [37], a FRAP method was carried out with slight modification. In wells of a 96-well plate, 20 µl (1000 µg/ml) of extract diluted appropriately in DMSO was mixed with 180 µl FRAP reagent, left for 6 minutes, and the absorbance was measured at 630 nm using ELISA reader ELx800 Absorbance Reader (Bio TekInstruments, Inc, USA). The FRAP values are expressed as micromoles of Ferrous equivalents (FE) per mg of the sample extract using the calibration curve constructed for different concentrations of FeSO4 (between 30-1000 µg/ml).

2.6.4. Antioxidant potency composite index (ACI)

The total antioxidant potency composite index was calculated according to [38] by giving all assays, DPPH, FRAP, and ABTS, an equal weight, giving the best score for each test an index value out of 100. Then, computing an index score for all other samples within the test as follows:

$$\text{Antioxidant Index Score} = \frac{\text{Sample Score}}{\text{Best Score}} \times 100$$

The mean of antioxidant tests for each extract was then taken as the value of antioxidant potency composite index (Table 2).

2.7. Statistical analysis

All statistics are expressed as the mean ± SE of three independent determinations. The significance levels for comparison of differences were determined with a one-way ANOVA at $P < 0.05$. The Pearson correlation test was employed to determine the correlation coefficients among bioactive compounds and different antioxidant assays. Principal Component Analysis (PCA) was used to better view the different data sets and the variability of the antioxidant activity of the different extracts used. This analysis was performed with the Minitab® 19.2020.1 software.

3. Results

3.1. Total phenolic content

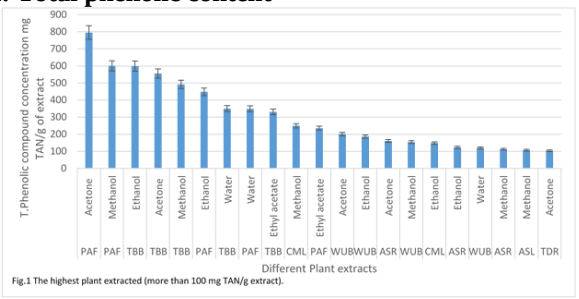

Figure 1 shows the total phenolic content of plant extracts (TPC). The plants extract had varying levels of total phenol. The highest levels were noted in *P. Africana*, especially with acetone extract (795.62 ± 0.74 mg/g), followed by the bark methanol extract (600.38 ± 0.62 mg/g).

The results showed that phenolics extracted were distributed in the extracting solvents in the following order: acetone > methanol > ethanol > ethyl acetate, respectively. Further, the plants with the highest phenolic content were in the order: *P. Africana*, *T. brownii*, *C. megalocarpus*, and W. *ugandensis*, respectively.

Table (2) summery of the best variable and ranking of it according to ACI.

Variable	T.phenol mg TAN/g extract	T.flavonoid mg Qu/g extract	DPPH IC_{50} µg/ml	FRAP µM Fe (II)	ABTS IC_{50} µg/ml	ACI %
TBB Acetone	555.66 ± 0.69	150.952 ± 0.71	36.81 ± 0.29	3769.64 ± 0.16	44.84 ± 0.27	92.1647 ± 0.32
PAF Acetone	795.62 ± 0.74	14.075 ± 0.73	50.37 ± 0.18	3449.65 ± 0.17	46.86 ± 0.19	79.2625 ± 0.24
TBB Methanol	492.07 ± 0.56	143.131 ± 0.64	72.33 ± 0.48	3009.67 ± 0.18	34.3 ± 0.19	76.9105 ± 0.19
PAF Methanol	600.38 ± 0.62	13.253 ± 0.56	55.38 ± 0.21	2926.04 ± 0.21	43.36 ± 0.30	74.3981 ± 0.31
PAF Ethanol	448.62 ± 0.53	142.655 ± 0.69	79.76 ± 0.27	2984.22 ± 0.18	48.86 ± 0.24	65.1720 ± 0.25
TBB Ethyl acetate	331 ± 0.42	82.233 ± 0.52	71.23 ± 0.54	2867.86 ± 0.22	57.83 ± 0.41	62.3558 ± 0.18
TBB Water	350 ± 0.48	109.472 ± 0.57	93.15 ± 0.37	2075.16 ± 0.21	40.63 ± 0.22	59.6622 ± 0.17
TBB Ethanol	598.57 ± 0.74	93.172 ± 0.79	158.69 ± 0.41	2838.77 ± 0.28	52.7 ± 0.32	54.5292 ± 0.21
PAF Water	349.17 ± 0.44	15.150 ± 0.62	146.23 ± 0.36	1449.73 ± 0.19	101.83 ± 0.41	32.4381 ± 0.34
WUB Ethan	187.33 ± 0.54	38.193 ± 0.43	171.88 ± 0.20	595.22 ± 0.31	101.31 ± 0.32	23.6875 ± 0.26
PAF Ethyl acetate	235.78 ± 0.37	16.129 ± 0.37	285.84 ± 0.42	1046.11 ± 0.34	160.29 ± 0.48	20.6758 ± 0.18
WUB Acetone	200.88 ± 0.68	47.377 ± 0.46	304.63 ± 0.30	649.77 ± 0.24	120.97 ± 0.52	19.2249 ± 0.16
WUB Methanol	154.7 ± 0.46	15.970 ± 0.81	333.78 ± 0.36	584.31 ± 0.27	221.83 ± 0.38	13.9970 ± 0.22
CMl Methanol	249.72 ± 0.46	37.233 ± 0.67	446.98 ± 0.34	457.04 ± 0.26	188.37 ± 0.42	12.8561 ± 0.20
TDL Water	10.3 ± 0.56	8.624 ± 0.34	5495 ± 0.66	49.79 ± 0.33	110.47 ± 0.36	11.0133 ± 0.19

3.2. Total flavonoid content

Figure 2 shows the total flavonoid content (TFC) of plant extracts. The extraction solvent affects the TFC of the plant extracts. The plants extract had varying levels of total flavonoid. The highest level of TFC was (150.951 ± 0.71) mg/g, noted in *T. brownii* acetone extract, followed by (143.13 ± 0.64) mg in *T. brownii* methanol extract and (142.655 ± 0.69) mg in *P. Africana* ethanol extract respectively.

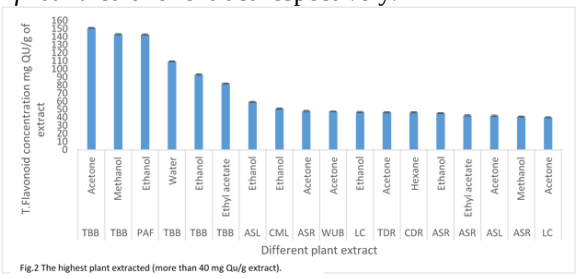

Fig.2 The highest plant extracted (more than 40 mg Qu/g extract).

The results showed that the most effective solvents were acetone, methanol, ethanol, and water. The highest plants in TFC were *T. brownii*, *P. Africana*, *A. secundiflora*, *C. megalocarpus*, *and w. ugandensis*, respectively

3.3. Antioxidant activity DPPH Assay

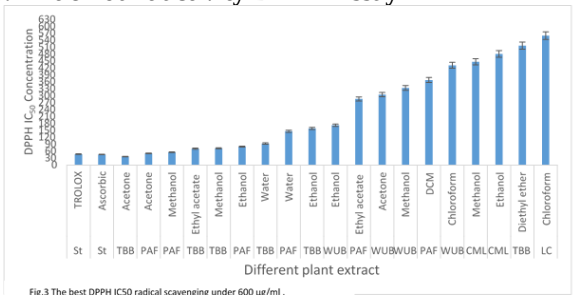

Fig.3 The best DPPH IC50 radical scavenging under 600 µg/ml .

Different sample extracts showed variability in their inhibitory activity against DPPH radical. A noticeable effect on radical scavenging activity was observed in figure (3). Amongst tested solvents, the highest radical scavenging activity was detected in acetone extract for bark of *T. Brownii*, followed by *P. Africana* IC_{50} (36.81± 0.29 µg/ml, 50.37 ± 0.18 µg/ml, respectively). IC_{50} for Ascorbic Acid (46.25 µg/ml) and TROLOX (47.4 µg/ml). The Diethyl ether extract of *Croton megalocarpus* bark showed very low radical scavenging activity with DPPH (2951.67 ± 0.55 µg/mL).

3.4. ABTS Assay

The sample results of ABTS radical scavenging activity of sample extracts are presented in figure 4. The IC_{50} (as TROLOX) was (68.33 µg/ml). While the best plant extract was bark of *T. Brownii* (methanol, water, then acetone) and IC_{50} values were (34.3 ±

0.19, 40.63 ± 0.22 and 44.84 ± 0.27 µg/ml, respectively), followed by *P. Africana* (methanol then acetone, ethanol) which was (43.36 ± 0.30, 46.86

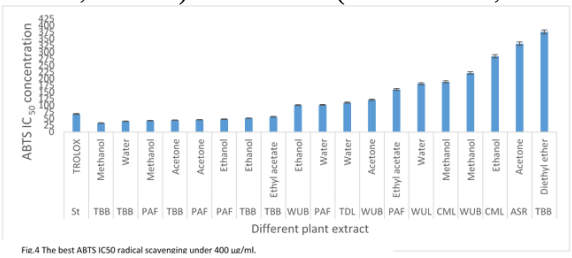

Fig.4 The best ABTS IC50 radical scavenging under 400 ug/ml.

± 0.19 and 48.86 ± 0.24 µg/ml, respectively). At the same time, the lower value was found in *Carissa edulis* root chloroform extract (3040.87 ± 0.84 µg/ml).

3.5. FRAP Assay

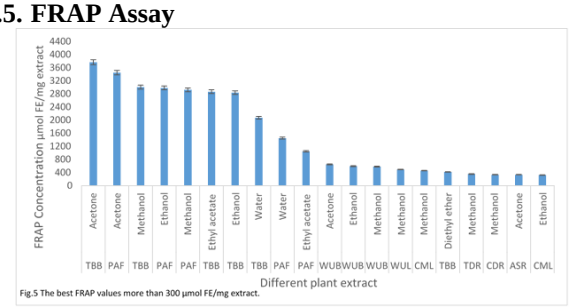

Fig.5 The best FRAP values more than 300 µmol FE/mg extract.

Results of reducing potential of extracts are demonstrated as FRAP values are summarized in figure 5. The highest FRAP values were obtained with acetone extract from the bark of *T. Brownii*, followed by *P. Africana* (3769.64 ± 0.16 µM Fe (II), 3449.65 ± 0.17 µM Fe (II), respectively).

The diethyl ether extract of *Croton megalocarpus* leaves showed meagre reducing potential (24.33 ± 0.78) µM Fe (II), compared to (4096 ± 0.31) µM Fe (II) Ascorbic acid at a concentration of 1 mg/ml for standard and investigate.

3.6. Antioxidant potency composite index (ACI)

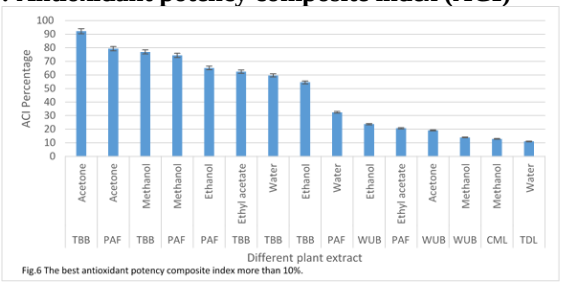

Fig.6 The best antioxidant potency composite index more than 10%.

DPPH, ABTS, and FRAP assay measured the antioxidant capacities of plant extract based on different mechanisms, leading to different rank orders for the antioxidant capacity of the same extract. The antioxidant potency composite index (ACI) was calculated to give equal weight to these entire methods. The ACI values showed in Table 2 and figure (6).

From the results, the highest values were obtained with acetone extract for the bark of T. Brownii, followed by P. africana (92.16 and 79.26, respectively), and Figure 6 showed that the ACI is more than 10%.

3.7. Principal Component Analysis (PCA)

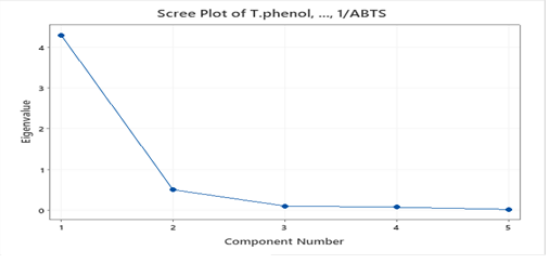

Fig.7 the scree plot figure.

The PCA was conducted on the mean values obtained from 117 plant crude extracts. From the scree plot figure (7), the PCA produced five components, the first two principal components accounting for 95.9% (85.8% and 10.1%, respectively) of the variance. So 95.9% of data variations could be described by two PCs the first principal component (PC1) revealed the highest eigenvalue (4.2912), The remaining four PCs (PC2, PC3, PC4 and PC5) yielded progressively lower eigenvalues (0.5040; 0.1068, 0.0044, 0.0763 and 0.0217, respectively) and did not explain the variability of the data.

The main dominant features for PC1 were total phenol, FRAP, 1/ABTS and 1/DPPH (0.459, 0.476, 0.467, and 0.459, respectively). While PC2's main dominant features were total flavonoid (-0.917).

3.8. Correlation between study variables

Pearson's correlation coefficient was conducted between the results of each pair of variables, DPPH, ABTS (IC_{50}) and was expressed as the reciprocal of the calculated IC_{50} values, Phenol, Flavonoid and FRAP normal value. In table (3) correlation matrix were calculated. As reported in Table(3) The highly positive correlation between total phenolic content and 1/DPPH, 1/ABTS, and FRAP ($r = 0.897$, $p<0.001$; $r=0.914$, $p<0.001$; and $r=0.954$, $p<0.001$ respectively); moderate positive correlation between total flavonoid content and total phenolic content, 1/DPPH, 1/ABTS, and FRAP ($r = 0.592$, $p<0.001$; $r=0.619$, $p<0.001$; $r=0.691$, $p<0.001$; and $r=0.683$, $p<0.001$ respectively) was observed.

A relatively higher positive correlation coefficient between FRAP and 1/DPPH; and 1/ABTS ($r=0.951$, $p<0.001$; and $r=0.949$, $p<0.001$ respectively); and positive correlation between 1/DPPH and 1/ABTS ($r=0.899$, $p<0.001$).

4. Discussion

Diabetes, rheumatoid arthritis, cardiovascular disease, atherosclerosis, neurological illnesses (Parkinson's, Alzheimer's, and Huntington's), cancer, and ageing have all been linked to oxidative stress [39], [40]. By scavenging free radicals, reducing lipid peroxidation, and other processes, plants may provide resistance to oxidative stress due to natural antioxidants such as phenolic acids and flavonoid compounds [41], [42].

Table (3) Correlation between study variables.

ALL		Flavonoid	FRAP	1/DPPH	1/ABTS
	Phenol	0.591972072	0.953618927	0.896935043	0.913683284
	p	2.07812E-12	8.48771E-62	1.44175E-42	8.75197E-47
	Flavonoid		0.682790341	0.619352348	0.691391004
	p		2.2717E-17	9.77558E-14	6.19417E-18
	FRAP			0.950722479	0.949981375
	p			2.54536E-60	5.87975E-60
	1/DPPH				0.899795849
	p				3.10607E-43

Crude plant extracts are natural multicomponent matrices comprising numerous biologically active compounds. Antioxidant compounds exert their effects, through different mechanisms, for example, transition metal ion chelation, disintegrating peroxides, electron acceptance, electron donation, and radical scavenging [43], [44]. As a result, the antioxidant activity of plant extracts cannot be accurately assessed using a single methodology [45]. Therefore, at least two testing methodologies are recommended to evaluate the antioxidant activity of crude plant extracts in vitro [46].

So, to examine the extract's overall antioxidant capability, DPPH, ABTS, and FRAP assays were used, each of which elicits a different mechanism of action. FRAP assay is based on electron transfer reaction, whereas DPPH and ABTS assays are based on electron and H atom transfer, and They may not necessarily measure the same activity [43], [44].

Thus, the current study was carried out with the goal of demonstrating the antioxidant potentials of eight medicinal plants known to be used in folk medicine in Kenya using three widely used methods (DPPH, ABTS, and FRAP), as well as establishing possible correlations between antioxidant activity and total phenolic and flavonoid contents of the extracts.

The observed antioxidant properties may vary according to extraction solvent polarity because it significantly influences extraction efficiency [47], [48].

PCA was applied to understand more about variables and variation between extracts based on their phenolic, flavonoid contents, and antioxidant activity (1/ABTS, 1/DPPH, and FRAP). This technique serves as a valuable tool for understanding much more about explaining results from the research study and the effects of different solvents on them.

Figure (8) revealed the outlier. The causes result for outlier are measurement error, wrong labelling, noise, or the most interesting sample [49]. In this case, we suggest the outlier is the most interesting sample because it's the same samples which have the highest ACI value

According to ACI and PCA analysis, the best overall antioxidant capacity is *T. Brownii* bark and *P. africana* bark (water, methanol, ethanol, ethyl acetate and acetone extract). Followed by *Warburgia ugandensis* bark (methanol, ethanol and acetone extract). Then *Croton megalocarpus* leaves ethanol extract, and at the end, *Tithonia diversifolia* leaves water extract., and this was in agreement with [50]–[54].

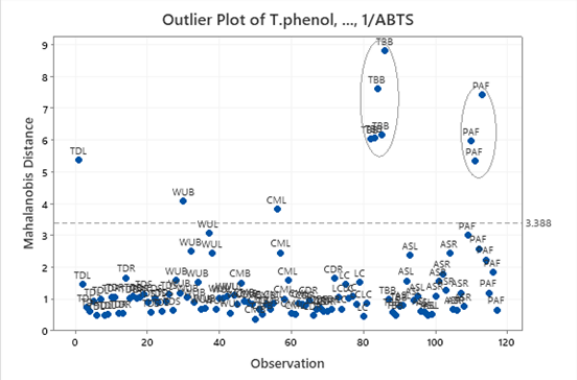

Fig.8 the outlier plot of PCA analysis.

According to Pearson's correlation coefficient, the three methods have a similar predictive capacity for antioxidant activity [55]. In our study, the agreement between the three assays probably indicates that these activities were mainly due to phenolics and flavonoids.

The highly positive correlation between total phenolic content and 1/DPPH, 1/ABTS, and FRAP suggests that phenolic compounds contribute significantly to the antioxidant activities of these species and, as a result, may play an essential role in the beneficial effects of these important medicinal plants. Several studies have found that phenolic compounds are major antioxidant constituents in selected plants and that there are direct relationships between their antioxidant activity and total phenolic content [56], [57].

Phenolics have strong antioxidant properties such as free radical scavenging, O2 scavenging, and Fe+2 chelation. Hydroxyl groups in phenolic compounds can donate hydrogen atoms, which can be combined with (reactive oxygen species) ROS and (reactive nitrogen species) NOS to end the oxidation reaction, preventing the generation of new radicals. Polyphenols with distinct properties are produced by the reaction of phenolic hydroxyl groups and benzene ring -electrons [58]. Furthermore, phenolics can chelate Fe+2 ions, which react with the free radicals

produced. Polyphenolics can strongly react with proteins and form hydrogen bonds due to the hydrophobic properties of the benzene ring. These properties allow phenolics to be antioxidant compounds and to inhibit the activity of free radical-generating enzymes such as some cytochrome P450 isoforms, lipoxygenases, cyclooxygenase, and xanthine oxidase [58], [59].

Flavonoids have a significant ability to directly scavenge ROS and trap free radicals as soon as they are generated through hydrogen donation or single-electron transferring. They have the ability to grab or bind metal ions in the human body, preventing them from being accessible for oxidation. Flavonoids can trap trace metal ions such as Fe2+ and Cu+ and play a key role in oxygen metabolism and the formation of free radicals. Flavonoids can act as an intracellular antioxidant by inhibiting the activity of enzymes involved in the generation of free radicals [60].

5. Conclusion

A comparative study on the methods of antioxidant capacity of 117 extracts of 8 Kenyan medicinal plants was performed. Different determination methods used *Terminalia brownii* and *Prunus africana* proved to have the highest mean values for all methods. Total phenolic content was positively correlated with FRAP, ABTS and DPPH values. Antioxidant potency composite index was calculated and based on the overall antioxidant index can be concluded that the acetone extract from *Terminalia brownii* bark had the highest antioxidant activity. and can be suggested as a potential natural source of antioxidants appropriate for utilization in nutritional/pharmaceutical fields. However, further evaluation of their bioactive compounds and antioxidant activities in living models is required.

6. Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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