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Original Article

Evaluation of the Antioxidant Activity of Nine Plants Used Medicinally by the Ilkisonko Maasai Community of Kenya

Julia Kimondo[1,*], Peggoty Mutai[1], Peter Njogu[2], Charles Kimwele[3]

ABSTRACT

Objectives: Many plants used in ethnomedicinal interventions have anti-inflammatory, antibacterial, antiviral, anti-aging and anticancer activities attributed to their antioxidant properties. The antioxidant properties may be attributed to high polyphenol content of the plants. The Ilkisonko Maasai are a pastoralist community from Kenya known to ingest certain select plant decoctions for prevention or treatment of targeted illnesses. This study evaluated the antioxidant properties of organic and aqueous extracts of these select plants. **Methods:** The antioxidant potential was evaluated by 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH) scavenging method. Total phenolic and flavonoid content were determined using the Folin–Ciocalteu's assay and aluminium chloride colorimetric test, respectively. **Results:** The organic extracts had significantly higher phenolic and flavonoid content than the aqueous extracts except for *Pappea capensis* in which the converse was observed. Among the studied plant extracts, *Acacia nilotica* had the highest phenolic content in the methanol and water extracts (237.26±1.83 mg and 149.66±0.60 mg tannic acid equivalent/g of extract) and the highest antioxidant activity in both the methanol and water extracts with an IC_{50} of 54.61µg/mL and 102.96µg/mL, respectively. The standard, ascorbic acid, had an IC_{50} of 50.32µg/mL. In both the methanol and water extracts, *Acacia reficiens* had the highest flavonoid content (130.62±1.78 and 99.80±1.73 mg catechin equivalents/g of extract, respectively). The correlation between the total phenolic content and antioxidant activity was statistically significant (Pearson's r= -0.841). **Conclusion:** This study found that select plants used by the Ilkisonko Maasai as medicinal plants exhibited high phenolic content and antioxidant activity, giving credence to their ethnomedicinal use.

Key words: Adaptogens, Aluminium chloride test, Bark, DPPH, Folin-Ciocalteu assay, Maasai, Polyphenols.

Julia Kimondo[1,*], Peggoty Mutai[1], Peter Njogu[2], Charles Kimwele[3]

[1]Department of Pharmacology and Pharmacognosy, University of Nairobi, Nairobi, KENYA.
[2]Department of Pharmaceutical Chemistry, University of Nairobi, Nairobi, KENYA.
[3]Department of Veterinary Anatomy and Physiology, University of Nairobi, Nairobi, KENYA.

Correspondence

Ms. Julia Kimondo
Department of Pharmacology and Pharmacognosy, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000 – 00200, Nairobi, KENYA.

Phone no: +254-704598539

E-mail: j_kimondo@yahoo.com

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INTRODUCTION

Oxidants are essential for normal cellular homeostasis and in aiding the immune system.[1] The oxidants exist in a delicate balance with the body's natural antioxidant systems. Oxidative stress occurs when oxidants overwhelm the physiological antioxidant systems causing cellular damage.[1] Oxidative stress is also known to affect multiple cellular processes leading to gene alterations, aberrant cell proliferation, abnormal metabolism and impaired cell cycles which have the effect of triggering neoplasms.[2] Diet rich in fruits and vegetables have been reported to reduce the progression of non-communicable diseases associated with oxidative stress such as cancer, cardiovascular diseases, diabetes, pulmonary disorders, Alzheimer's disease and other degenerative ailments.[3] This is primarily due to natural antioxidative plant polyphenols which act as reducing agents thereby protecting the body against oxidative damage.[4] Polyphenols have been shown to improve health not only due to their antioxidant property but also as modulators of signaling pathways involved in the progression of cardiovascular disease.[5] Polyphenols comprise of a vast number of compounds with one or more aromatic rings having one or more hydroxyl groups attached to them.[6] Examples are phenolic acids, flavonoids, anthocyanins, lignans and stilbenes.[7] Generally, these natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, such as anti-inflammatory, antibacterial, antiviral, anti-aging and anticancer.[4] Flavonoids and phenolic acids are the most commonly encountered dietary polyphenols.[7] Plant phenolics occur as secondary metabolites and are generally involved in protecting the plant against pathogens, animal and insect attacks, ultra violet radiation, as well as contributing to the plants colours.[4]

The Ilkisonko Maasai are an indigenous pastoralist community that reside in the southern part of Kenya. They are still heavily steeped in their traditional ways of life, a reflection of which is their continued contemporary use of ethnomedicinal healthcare systems. These practices include dietary use of decoctions made from roots and stem barks of certain plant species prevalent in their locality as food

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additives for general wellbeing or administration for medical use in prevention and treatment of various illnesses and occasionally as adaptogens.[8] Plants used as adaptogens have high levels of phenolic compounds which act as antioxidants, anti-inflammatory, antidepressants and may regulate aging.[9] This study assesses the phenolic content, flavonoid content and the antioxidant activity of nine plant species (Table 1) used ethnomedicinally by the Ilkisonko Maasai community identified during a previously reported ethnobotanical survey.[10] This evaluation is part of research efforts geared towards giving scientific credence to ethnomedicinal systems of treatment among indigenous Kenyan communities and may herald the discovery of novel naturally-occurring antioxidant compounds.

MATERIALS AND METHODS

Materials, reagents and equipment

Methanol, ascorbic acid standard, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Folin Ciocalteu's phenol reagent, tannic acid ACS, sodium nitrite ($NaNO_2$) analytical reagent, catechin analytical standard, sodium carbonate (Na_2CO_3) powder, sodium hydroxide (NaOH) pellets and aluminium chloride hexahydrate ($AlCl_3.6H_2O$) powder were obtained from Sigma Aldrich Co. (St Louis, MO, USA).

Shimadzu 1800 UV-Vis spectrophotometer was used for all spectrophotometric determinations while Büchi Rotavapor R-200 rotary evaporator was used to evaporate extracting solvents from the plant extracts to complete dryness.

Plant selection

The procedures used in plant collection are described in detail by Kimondo *et al*.[10] Briefly, ethnobotanical knowledge of food and medicinal plants of the Ilkisonko Maasai community was obtained through interviews conducted among herbal practitioners in Loitokitok Sub-County, Kenya. A total of 30 plants were encountered in the ethnobotanical survey and nine among them (Table 1) namely: RN- *Rhus natalensis*, AD- *Acacia drepanolobium*, AN- *Acacia nilotica*, AR- *Acacia reficiens*, AB- *Acacia robusta*, GV- *Grewia villosa*, XA- *Ximenia americana*, RP- *Rhamnus prinoides* and PC- *Pappea capensis*, were selected for further analysis due to their high antioxidant activity detected following an initial antioxidant screening.

Extraction

The medicinal plants identified were collected, catalogued and deposited in the University of Nairobi herbarium.[10] The part of the plant traditionally used was dried under shade at ambient temperature and pulverized to fine powder. The methanol extract was obtained by macerating the powder in six times its weight of 80% v/v methanol at room temperature for 72 h. The extracts were filtered, concentrated using a rotary evaporator and dried further in an oven at 40°C for 24h. Water extracts were obtained by heating the powder to 60°C in 10 times its weight of distilled water for 20 min. The extracts were then filtered and concentrated using a freeze drier. The dry extracts were stored under refrigeration at 4°C awaiting phytochemical screening.[19]

Phytochemical screening

Total phenolic content

The total phenolic content of the extracts was determined using the Folin and Ciocalteu's method. Each extract was dissolved in distilled water to give a concentration of 0.5 mg/mL. A 0.5 mL aliquot of the extract was added to 2.5 mL of 1N Folin–Ciocalteu reagent. The mixture was then shaken and allowed to stand for 6 min, before addition of 2.0 mL of 7.5%

Table 1: Plants used in the assay (Partially adopted from a previously reported ethnobotanical study)[10]

Taxonomic name (Family)	Code	Uses	Part used in the assay	No. of mentions	Chemical constituents/ Reported Pharmacological activity
Rhus natalensis Krauss (Anacardiaceae)	RN	Strengthener, respiratory disorders, stomachic, malaria	Root bark	3	A flavanone found in Rhus spp. has *in-vitro* anti-inflammatory activity.[11]
Acacia drepanolobium Harms ex Sjöstedt (Fabaceae)	AD	Retained placenta in cows, postpartum pain in humans, fertility, tonic	Stem bark	15	Presence of tannins and proanthocyanidins which are anti-carcinogenic.[12]
Acacia nilotica (L.) Willd. (Fabaceae)	AN	Strengthener/ tonic, appetizer, body aches, stomachic, stamina, stimulant/ excitant	Stem bark	20	Niloticane has anti-inflammatory and antibacterial effect.[13]
Acacia reficiens subsp. *misera* (Vatke) Brenan (Fabaceae)	AR	Strengthener, appetizer, tonic/ adaptogen, laxative	Root bark	16	No reported activity
Acacia robusta Burch. (Fabaceae)	AB	Retained placenta in cows and humans	Stem bark	22	The methanol extract exhibited antifungal activity.[14]
Grewia villosa Willd. (Malvaceae)	GV	Food, galactagogue, strength/ tonic, stomach ache in kids	Stem bark	25	The root contains harman alkaloids and possesses antitumor activity.[15]
Ximenia americana L. (Olacaceae)	XA	Stomach-ache in children, food, tonic, constipation	Stem and leaves	25	Leaves contain sambunigrin, gallic acid, gallotannins and flavonoids (quercetin). Polysaccharides rich fractions have anti-inflammatory activity.[16]
Rhamnus prinoides L. (Rhamnaceae)	RP	Sexually transmitted infections, back and joint aches, arthritis, aids in digestion, tonic	Root bark	16	Extract contain laxative anthraquinones and has antiplasmodial activity.[17]
Pappea capensis Eckl. & Zeyh. (Sapindaceae)	PC	Strengthener, food, fertility, stomach ache, stamina	Stem bark	17	It contains quercetin-3-*O*-rhamnoside and epicatechin which have anti-inflammatory effect.[18]

w/v Na_2CO_3. The solution was then adjusted with distilled water to a final volume of 6 mL and mixed thoroughly. After incubation in the dark for 2h, absorbance at 765 nm was determined spectrophotometrically versus a blank containing only 1N Folin–Ciocalteu reagent and 7.5% w/v Na_2CO_3. The total phenolic content of the extracts were expressed as milligrams of tannic acid equivalents per gram of extract (mg TAE/g E) from a calibration curve prepared simultaneously using tannic acid standard (50-250 µg/mL).[20] All samples were analysed in triplicate.

Flavonoid content

The aluminium chloride colorimetric method was used for the determination of the total flavonoid content of the extracts. In estimating the flavonoid content, aluminium chloride forms acid stable complexes with the hydroxyl groups of flavones and flavonols. Catechin was used to make the standard calibration curve. Standard solutions (6.25-100 µg/mL) of catechin were prepared by serial dilutions in methanol. The extracts were dissolved in methanol to give a concentration of 0.5 mg/mL. A 0.5 mL aliquot of the standard solution or test extract was mixed with 0.75 mL of 5% w/v $NaNO_2$. After 5 min, 0.15mLof 10% w/v $AlCl_3$ was added and 6 min later, 0.5 ml of 1M NaOH was incorporated into the mixture. The solution was then adjusted with distilled water to a final volume of 3 mL, mixed thoroughly and incubated for 60 min at room temperature. The absorbance of the reaction mixture was then measured spectrophotometrically against a blank at 510 nm. The blank constituted all other reagents with distilled water added as a replacement for the sample/standard. The concentration of total flavonoid content in the test sample was calculated from the calibration plot and expressed as milligram of catechin equivalent per gram of extract (mg CE/g E).[21] All the determinations were carried out in triplicate.

Antioxidant activity

The assay for antioxidant activity was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as the oxidant described by Sánchez-Moreno *et al*.[22] The test solutions of the plant extracts were prepared in 95% v/v methanol for the methanol extracts and in distilled water for the water extracts, to obtain concentrations ranging from 12.5-400 µg/mL. Ascorbic acid was used as a standard in concentrations ranging from 3.125-100 µg/mL. The oxidant was prepared by dissolving 3.94 mg of DPPH in 100 mL of 95% v/v methanol. This solution was prepared just before use and stored in the dark to minimize degradation. A 200µL aliquot of the test/standard solution was placed in a vial and 2800µL of DPPH added. The mixture was kept in the dark for 30 min and optical density measured spectrophotometrically against a blank at 517 nm.[22] The assay was done in triplicate.

The antioxidant activity of the test extracts was expressed as IC_{50} (inhibitory concentration), the concentration (expressed in µg/mL) of sample required to cause a 50% reduction in DPPH radicals. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph that plotted inhibition percentage against concentration.

Inhibition of free radical of DPPH in percentage terms (I %) was calculated as shown in Equation 1.

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100 \quad \text{Equation 1}$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the sample) while A_{sample} is the absorbance of the test extract (or the standard).

Statistical Analysis

All the experiments for determination of total phenolic content, flavonoids content and antioxidant activity were conducted in triplicate. The values are expressed as the mean ± standard deviation (SD). The Statistical Package for the Social Sciences (SPSS) version 16 was used for statistical analysis of the results. Differences between the two contents or activity of the extracts were determined using the paired *t*-test. A *p* value less than 0.05 ($p<0.05$) was considered statistically significant. Correlation between total phenols and total flavonoid content and antioxidant activity was determined using Pearson's chi-squared test.

RESULTS

Phytochemical screening

The total phenolic content in the extracts as assayed by the Folin-Ciocalteu's reagent is represented in Figure 1. In eight of the nine plants assessed, the methanol extracts had a higher phenolic content compared to the water extracts though only six exhibited significant difference. *Pappea capensis* was an exception where the phenolic content in the water extract is higher at 56.71±0.26 mg and 124.15±0.70 mg TAE/g of extract for methanol and water extracts, respectively. Methanol extracts for RN, AD, AN, AR, AB and PC showed significant difference ($p<0.05$) in the total phenolics extracted when compared to the water extracts. In the methanol extracts, *A. nilotica* had the highest phenolic content (237.26±1.83) while *P. capensis* had the lowest (56.71±0.26) mg TAE/g of extract. The total phenolic content for the methanol extracts was ranked as follows: AN>AB>XA>AR>RN>GV>AD>RP>PC. In the water extracts, *A. nilotica* also had the highest (149.66±0.60) while *R. prinoides* had the lowest phenolic content (95.31±0.73) mg TAE/g of extract. The total phenolic content for the water extracts was ranked in the order: AN>XA>RN>AR>AD>PC>GV>AB>RP.

The flavonoid content was higher in the methanol extracts than the water extract with the exception of *P. capensis* where the converse was observed (Figure 2). All the plant extracts analysed showed significantly higher ($p<0.005$) total flavonoid content in the methanol compared to the water extracts. In both the methanol and water extracts, *A. reficiens* had the highest (130.62±1.78 and 99.80±1.73 mg CE/g of extract, respectively) while *A. drepanolobium* had the lowest flavonoid content (35.12±0.59 and 16.53±0.18 mg CE/g of extract, respectively). The flavonoid content for the methanol extracts was ranked as such: AR>GV>XA>AN>RN>AB>RP>PC>AD while that of the water extracts was: AR>GV>XA>AN>PC>RN>RP>AB>AD.

Antioxidant activity of plant extracts

The DPPH assay for antioxidant activity is based on the ability of the antioxidant to scavenge the DPPH cation radical and ascorbic acid is used as a standard. The IC_{50} value is used to indicate degree of antioxidant activity and it is defined as the concentration of substrate that causes 50 % reduction in the DPPH colour. The lower the IC_{50} value, the higher the antioxidant activity.[23] For each particular plant, the antioxidant activity of the methanol extract was much higher than that of the water extract with the methanol extract exhibiting at least 1.5 to 3 times higher antioxidant activity compared to the water extract except for *P. capensis* where the contrary was observed. *Acacia nilotica* had the highest antioxidant activity in both the methanol and water extracts with IC_{50} values of 54.61 µg/mL and 102.96 µg/mL, respectively. This was close to the standard Ascorbic acid which had an IC_{50} value of 50.32 µg/mL. *Pappea capensis* had the lowest antioxidant activity (378.62 µg/mL) in the methanol extracts while *R. prinoides* had the lowest antioxidant activity (377.27 µg/mL) in the water extracts. The antioxidant activity for the methanol extracts, from the highest to the lowest, was ranked as follows: AN>AB>XA>GV>AR>AD>RN>RP >PC while that of the water extracts was as follows: AN>XA>RN>AR>AD>PC>GV>AB>RP.

Table 2 shows the linear correlation (Pearson's) between the three assays. There is a significant negative correlation between IC_{50} (whose measure is the reciprocal of the antioxidant activity) with phenolic content

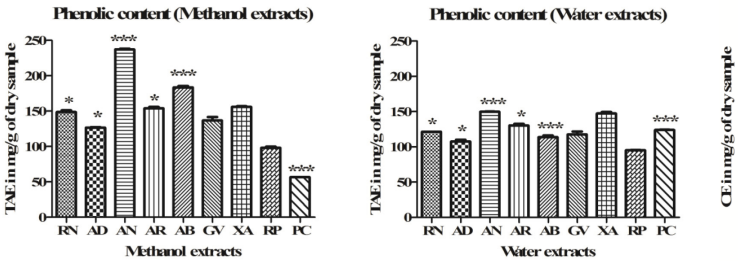

Figure 1: Total phenolic content in the studied plant extracts. RN- *Rhus natalensis*; AD- *Acacia drepanolobium*; AN- *Acacia nilotica*; AR- *Acacia reficiens*; AB- *Acacia robusta*; GV- *Grewia villosa*; XA- *Ximenia americana*; RP- *Rhamnus prinoides*; PC- *Pappea capensis*; TAE- Tannic acid equivalents. Each value is expressed as mean ± standard deviation (SD). The phenolic content in the methanol and water extracts showed statistically significant differences (* $p < 0.05$; *** $p < 0.001$).

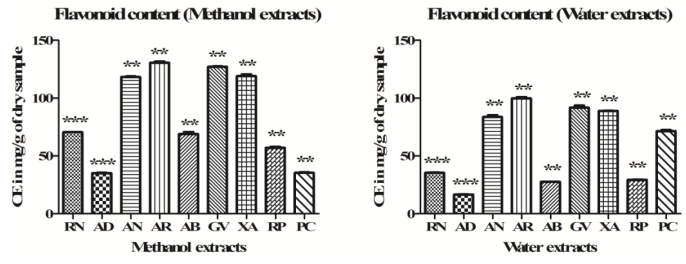

Figure 2: Total flavonoid content in the studied plant extracts. RN- *Rhus natalensis*; AD- *Acacia drepanolobium*; AN- *Acacia nilotica*; AR- *Acacia reficiens*; AB- *Acacia robusta*; GV- *Grewia villosa*; XA- *Ximenia americana*; RP- *Rhamnus prinoides*; PC- *Pappea capensis*; CE- Catechin equivalents. Each value is expressed as mean ± SD. The flavonoid content in the methanol and water extracts showed statistically significant differences (** $p < 0.005$; *** $p < 0.001$).

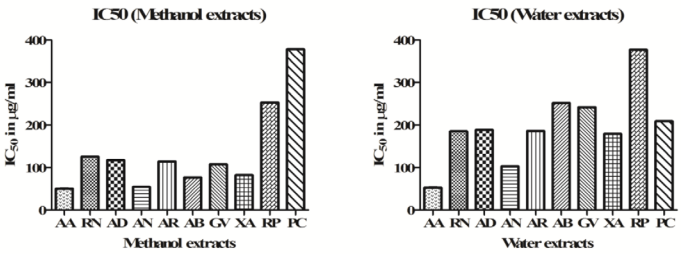

Figure 3: Antioxidant activity of the studied plant extracts. RN- *Rhus natalensis*; AD- *Acacia drepanolobium*; AN- *Acacia nilotica*; AR- *Acacia reficiens*; AB- *Acacia robusta*; GV- *Grewia villosa*; XA- *Ximenia americana*; RP- *Rhamnus prinoides*; PC- *Pappea capensis* while AA- Ascorbic acid was used as the control.

(r=-0.841) and flavonoid content (r=-0.597), with that of phenolic content being higher. The correlation coefficient between the total phenolic content and flavonoid content is significantly higher (r=0.621, $p < 0.05$) than that between antioxidant activity and flavonoid content (r=-0.597, $p < 0.05$).

DISCUSSION

The Folin-Ciocalteu's reagent measures the reducing capacity of phenols to give the total phenolic content in a sample.[6] Thus, the Folin- Ciocalteu's method is also considered an alternative antioxidant capacity assay because its basic mechanism is oxidation/reduction reaction.[20] Phenolics have strong antioxidant activity through chelation and free radical scavenging activity on mostly hydroxyl and peroxyl radicals. The methanol extracts had higher levels of phenolic content than the water extracts with the exception of *P. capensis*. This is consistent with other studies where methanol has been found to be more efficient in extraction of polyphenols.[6] *Acacia nilotica* had the highest phenolic content and antioxidant activity. This is in agreement with Aadil[24] where the methanolic fraction of *A. nilotica* had the highest polyphenol content in the bark and leaves. Further, Panossian and Wikman[9] also showed that phenolics contribute to the adaptogenic effect of some plants. Findings from our study indicate that eight of the nine studied plants (except for *A. reficiens*) that were used as adaptogens are high in total phenols.[10] This could explain why the eight plants have been routinely used as adaptogens by the Ilkisonko Maasai as they may derive their beneficial health effects from the observed high antioxidant activity.

Flavonoids act as antioxidants by stabilizing reactive oxygen species, an activity heavily influenced by the structure.[25] The flavonoid content was higher in the methanol extracts than in the water extracts except for *P. capensis* where the converse was true. This is similar to the pattern seen in phenolic content and in other studies where aqueous alcoholic extraction yields a higher quantity of polyphenols.[6] The poor solubility of flavonoids in water may explain the difference in their concentration between the water and aqueous alcoholic extracts.[26] The Ilkisonko Maasai prepare most of their decoctions in water before adding them to soups or milk. This study indicates that aqueous alcoholic extraction of polyphenols is more efficient than aqueous extraction. The plants in this study have also been used in illnesses of whose aetiology is microbial such as sexually transmitted infections, stomach ache and respiratory disorders.[10] Plants with antimicrobial activity are characterized by high phenolic and flavonoid content and consequently the high antioxidant activity which could be the basis for this antimicrobial activity.[27] There were variations in the polyphenol content in plants from the same genus (*Acacia* spp.) which could be due to intrinsic genetic species differences or differences

Table 2: Comparison between different assays represented by correlation coefficient.

	Correlation coefficient (r) n= 18		
Variable	Phenolic content	Flavonoid content	Antioxidant activity
Phenolic content	1	0.621* ($p < 0.05$)	-0.841*** ($p < 0.001$)
Flavonoid content	0.621* ($p < 0.05$)	1	-0.597* ($p < 0.05$)
Antioxidant activity	-0.841*** ($p < 0.001$)	-0.597* ($p < 0.05$)	1

Significant correlation * $p < 0.05$; *** $p < 0.001$

in environmental factors, time of collection and/or storage conditions.[28] It has been previously reported that the amount of phenolics may vary considerably in some plants due to geographical variations and environmental factors such as humidity and, temperature as well as leaf aging which invariably influence the antioxidant activity.[29]

With a few exceptions, the phenolic content closely mirrored the antioxidant activity. Previous studies have shown the antioxidant activity to be higher in solvents with lower polarity as they dissolve higher molecular weight phenols giving the extract stronger antioxidant effects.[29] The observed negative correlation between the total phenolic/flavonoid content with the antioxidant IC_{50} value is consistent with a research by Farasat *et al*.[30] which shows that the higher the phenolic/flavonoid content, the lower the IC_{50} value and hence the higher the antioxidant activity per gram of extract. Other studies have confirmed the direct relationship between phenolic content and antioxidant activity[28] and this lends credence that the observed antioxidant activity in the studied plants may be largely due to the phenolic content in the extracts. The significant negative correlation between antioxidant activity and flavonoids (r=-0.597) implies that although the flavonoids may contribute to the antioxidant activity, they lack the structural advantage of being strong antioxidants.[25] Nevertheless, it could plausibly be assumed that there are other compounds that also contribute to the antioxidant activity.

CONCLUSION

With the exception of *Rhus natalensis,* eight of the nine plants included in this study were commonly used by the Ilkisonko Maasai for ethnomedicinal interventions. This study shows that these commonly used medicinal plants are characterized by high phenolic content, high flavonoid content and attendant elevated antioxidant activity. This affirms the importance of using ethnobotanical approach in search of new phytochemicals with potential for application in human therapy. The community and ultimately the wider society could benefit from the findings of this study by targeted use of these medicinal plants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS

DPPH: 1,1-diphenyl-2-picrylhydrazyl; **TAE:** Tannic acid equivalents; **CE:** Catechin equivalents; **IC:** Inhibitory concentration; **SD:** Standard deviation; **RN:** *Rhus natalensis*; **AD:** *Acacia drepanolobium*; **AN:** *Acacia nilotica*; **AR:** *Acacia reficiens*; **AB:** *Acacia robusta*; **GV:** *Grewia villosa*; **XA:** *Ximenia americana*; **RP:** *Rhamnus prinoides*; **PC:** *Pappea capensis* while; **AA:** Ascorbic acid.

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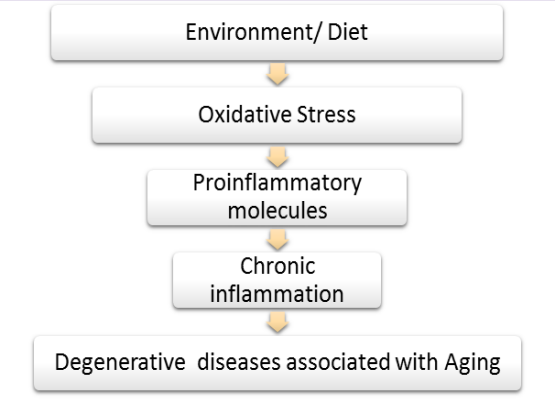
GRAPHICAL ABSTRACT



SUMMARY

- Most of the plants included in this study were commonly used by the Ilkisonko Maasai for ethnomedicinal interventions. This study shows that these commonly used medicinal plants are characterized by high phenolic content, high flavonoid content and attendant elevated antioxidant activity. Plants used as adaptogens have high levels of phenolic compounds which act as antioxidants, anti-inflammatory, antidepressants and may regulate aging.

ABOUT AUTHORS

Julia Kimondo, completed Masters in Materia Medica (Pharmacognosy) from Tianjin University of Traditional Chinese Medicine. She is currently a PhD student (Pharmacognosy) in the University of Nairobi. Her research interest are in using herbs to manage metabolic syndrome and inflammation disorders. Also in phytochemistry.

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