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Phytochemical Screening, Antioxidant Activity and Inhibitory Potential of Five Kenyan Medicinal Plants

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Authors' contributions

This work was carried out in collaboration among all authors. Author MDW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AC, MC, M-MD managed the analyses of the study and reviewed the study design and all drafts of the manuscript. Author IJ managed the literature searches and laboratory work. All authors read and approved the final manuscript.

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ABSTRACT

The present study was conducted to evaluate preliminary phytochemical analysis and *in vitro* antioxidant activities of five plants (*Olea europaea, Kigelia africana, Terminalia mollis, Croton macrostachyus and Bridella micrantha* extracts). The plants were collected from Baringo County in Kenya, dried, pulverized into fine powders and extracted using methanol. Phytochemical analysis showed the presence of alkaloids, aminoacids and proteins, flavonoids, saponins, steroids, tannins and triterpenoids. The root extracts were further investigated for their potential antioxidant activity by using radical scavenging DPPH (2, 2-Diphenyl-2-picryl-hydrazyl) technique. Methanol extract of

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roots from the plants showed significant differences in DPPH radical scavenging activities. The results were concluded that extracts have a more secondary metabolites and potential source of antioxidants, which is warranty to evaluate further *in vivo* pharmacological studies.

Keywords: Antioxidant activity; bridella micrantha; croton macrostachyus; Kigelia Africana; olea europaea; terminalia mollis; secondary metabolites.

1. INTRODUCTION

The flora of the tropical region especially Kenya exhibit remarkable diversity and a rich sources of medicinal plants [1]. The crude extracts of these plants have been used for a long time in traditional folklore medicine [2-4]. In Kenya there are large diverse of plants key of which are *Olea europaea* (Family: Oleaceae), *Kigelia africana* (Family: Bignoniaceae), *Terminalia mollis* (Family: Combretaceae), *Croton macrostachyus* (family: Euphorbiaceae) and *Bridelia micrantha* (Family: Phyllanthaceae [formerly Euphorbiaceae]) [5-9].

The therapeutic success of fruits and leaves *of O. europaea* in traditional medicine result in reduced blood sugar, cholesterol, uric acid, whilst treating diabetes, hypertension, inflammation, diarrhea, respiratory and urinary tract infections, asthma, hemorrhoids, and rheumatism. Extracts of *Kigelia africana* is traditionally used to treat stomach-ache and diarrhea; the fruit is used for the treatment of female sterility [10]. Pounded provent gonorrhea and impotence while fresh leaves are chewed and swallowed against abdominal pain [11]. Meanwhile the extracts of *Terminalia mollis* have a long history of usage in traditional medicinal systems [12-14]. Crude extracts of *Croton macrostachyus* (family: Euphorbiaceae) has a variety of medicinal uses [15,16] *ridelia micrantha*, as an anti-helmintic, anti-anemic, antibacterial, anti-diabetic, anti-diarrhoeal, anti-inflammatory, anti-malarial, antinociceptive, antiviral, hypoglycemic and for abdominal pain and cardiovascular diseases [17].

The pharmacological properties of these plant species are probably due to the presence of plant secondary metabolites, which contains several bioactive compounds [18]. Polyphenols [19,20] flavonoids [21], tannins [22,23], organic acids [24], coumarins [25], and carotenoids [26] have the potency to inhibit the oxidative mechanisms against various disease [27]. These compounds act as antioxidants by different ways: as reducing agents, hydrogen donators, free radical's scavengers, and singlet oxygen quenchers [28,29]. Despite the folklore uses and the phytochemical studies in other geographical locations worldwide, the plants in this study have rarely been evaluated for antioxidant activity and inhibitory potential. Since the metabolite composition is affected by the local environment, there is need to examine the phytochemical compositions, antioxidant activity and inhibitory potential of these local plants that have diverse use in local medicine in the Sub Saharan Africa.

In light of the scanty data on the roots of the herbal medicine especially in the tropical regions where there are large forested land under these plants, the aim of this study was to evaluate the phytochemical compounds in *O. europaea*, *K. africana, T. mollis, C. macrostachyus* and *B. micrantha* and determine their antioxidant activity and inhibitory potential.

2. MATERIALS AND METHODS

2.1 Sources of Plant Extracts

Five plants species: *O. europaea*, *K. africana, T. mollis, C. macrostachyus* and *B. micrantha* were collected from Baringo County in Kenya and preserved in cool boxes to before laboratory extraction and analyses. The voucher specimen were taken to the herbarium of the Museums of Kenya for authentication. The plant extracts were then taken to the KEMRI Nairobi for methanolic extraction.

2.2 Sample Preparation and Extraction of Compounds of Plant Species

The roots were cut into small pieces and air-dried for three weeks under a shed. The dried specimens were shred using an electrical mill in readiness for extraction. Cold sequential extraction were carried out on plant material with analar grade methanol [30], where six hundred milliliters of methanol were added to 300 g of the shred specimen and flasks placed on a shaker and soaked for 48 h. The residue were filtered using a Buchner funnel under vacuum until the sample dry. The filtrate will then be concentrated under vacuum by rotary evaporation at 30 - 35°C [31]. The concentrate were transferred to a

sample bottle and dried under vacuum; the weight of the dry extract were recorded and stored at -20°C until required for bioassay.

2.3 Phytochemical Analysis

All the extracts (0.05 g/ml) were subjected to preliminary phytochemical screening following standard methods [32]. In general, tests for the presence or absence of phytochemical compounds involved the addition of an appropriate chemical agent to the preparation in a test tube. The mixture was then vortexed. The presence or absence of compounds were subsequently detected.

2.4 Determination of DPPH free Radical Scavenging Activity

The ability of the selected plant extracts to scavenge DPPH free radicals was estimated by the reduction of the color reaction between DPPH solution and sample extracts. Briefly, 2 mL of 0.12 mM solution DPPH in methanol was added to 1 mL of various concentrations of each extract (50 - 1000 µg/mL) to be tested. After 30 min at room temperature, the absorbance of the reaction mixture was measured at 517 nm using a spectrophotometer. Ascorbic acid (2- 20 µg/mL) was used as positive controls.

The scavenger activity was calculated as follows:

I% = (A Control-A Sample)/A Control) * 100. Where A Control is the absorbance of the blank sample (t = 0 min) and A Sample is the absorbance of the test extract or standard (t = 30 min). The tests were carried out in triplicate.

The IC_{50} values (concentration in µg/mL required to inhibit DPPH radical by 50%) were estimated from the percentage inhibition versus concentration plot, using Probit methods. The data were presented as mean values ± standard deviation (n = 3)

2.5 Statistical Analyses

All statistical analyses will be performed with STATISTICA 6.0 (Sta Soft, 2001) statistical package. The normality of the data must be verified by hypothesis tests (Shapiro-Wilk tets to determine the overall test applicable in the data. The experimental data obtained from the antioxidant activity assays were expressed as mean and standard deviation. To evaluate statistical differences, One-way ANOVA and student's t-test were used. The comparison between the averages was performed through the Duncan's Multiple range test (DMRT) to measure specific differences between pairs of means. *P* values ≤ 0.05 were considered statistically significant.

3. RESULTS

3.1 Phytochemical Screening of Plant Extracts

The phytochemical analysis conducted on the extracts revealed the presence of tannins, flavonoids, steroids phlobatannins, cardiac glycoside, terpenoids and saponins (Table 1). The *K. africana* and *Olea europa* had the largest number of phytochemical compounds. Major compounds like polyphenols, alkaloids, flavonoids, cumarins, anthocyanins, trepenoids, saponins and tannis have been observed in thousands of medicinal plants [33-35]. Some screening compounds of our preliminary phytochemical analyses have been reported previously [36]. The phytochemicals in these plants contain biological activities of the plant extracts against a range of parasites.

3.2 DPPH Radical Scavenging Activity

The abilities of different phenolic compounds from different plant extracts assayed to scavenge to the DPPH+ free radical under defined conditions is provided in Fig. 1. The DPPH test showed an increase of the antioxidant activity in the order: *T. mollis* > *C. macrostachyus* > *K. Africana* > *M. micrantha* > *O. europeae*. The *T. mollis* root extracts showed the highest DPPH radical scavenging activity. Furthermore, this activity increase progressively by increasing the concentration of extracts, this observed activity was dose-dependent. Diverse radical scavenging activity has been reported for *O. europaea*, *K. africana, T. mollis, C. macrostachyus* and *B. micrantha* [37-41]. This requires determination of measures such as antioxidant and inhibitory potential [42,43]. The oxidative stress has been implicated in numerous diseases whose solution lies in the investigation of antioxidant properties, which may offer resistance against oxidative stress by scavenging free radicals and inhibiting lipid peroxidation [44]. The obtained results are in concordance with others reported previously [45-47]. The present work suggests a strong correlation between antioxidant activities and a

high content of phenols, which means that phenols compounds are the main agents responsible and contribute largely in the antioxidant activities of medicinal plants. Furthermore, the anti-radical ability of phenolic compounds is due to their capacity to trap free radicals through the transfer of the hydrogen atom then transformed into a stable molecule, and their reducing power is due to the presence of hydroxyl group in their structure that can serve as an electron donor [48,49].

The % inhibition, IC_{50} and IC_{90} of the tested plant extracts are shown in Table 2. There were significant differences in the optimal efficacy of the test drugs ($P < 0.05$). The most effective % inhibition, IC_{50} and IC_{90} of the five plant extracts was *T. mollis* followed by *C. macrostachyus* while *O. europeae* was the least effective. Growth inhibition activity of the plant extracts is attributed to their ability to bind ergosterol in the parasite membrane or sequester cholesterol in the host membrane, thereby inhibiting the macrophage-parasite interaction which is necessary for macrophage infection [50]. The activity of *T. mollis* is attributed to punicalagin, ellagic acid and their derivates [51,52,53]. These compounds also have high solubility in methanol and could therefore be in large quantitative in the present sample. *T. mollis* extract also contain other active compounds including urolithins and benzopyranones, which are cystein protease inhibitors [54,14,55].

3.3 Reducing Power (FRAP) of Different Plant Extracts

The reducing power assay (FRAP) of studied plant extracts was investigated and the results are given in Fig. 2. The results obtained shows that our extracts had a potency reducing power. In addition, *T. mollis* extract showed a higher absorbance followed by *C. macrostachyus* while the least absorbance occurred in *O. europeae* extract. The observed reducing power of both *T. mollis, C. macrostachyus*, *K. africana* and *B. micrantha* were dose dependent and increased with increasing amounts of extracts. However, the reducing power of *O. europeae* showed lower level of increase after 200 mg/l. The FRAP assays of our extracts have demonstrated an antioxidant potency,

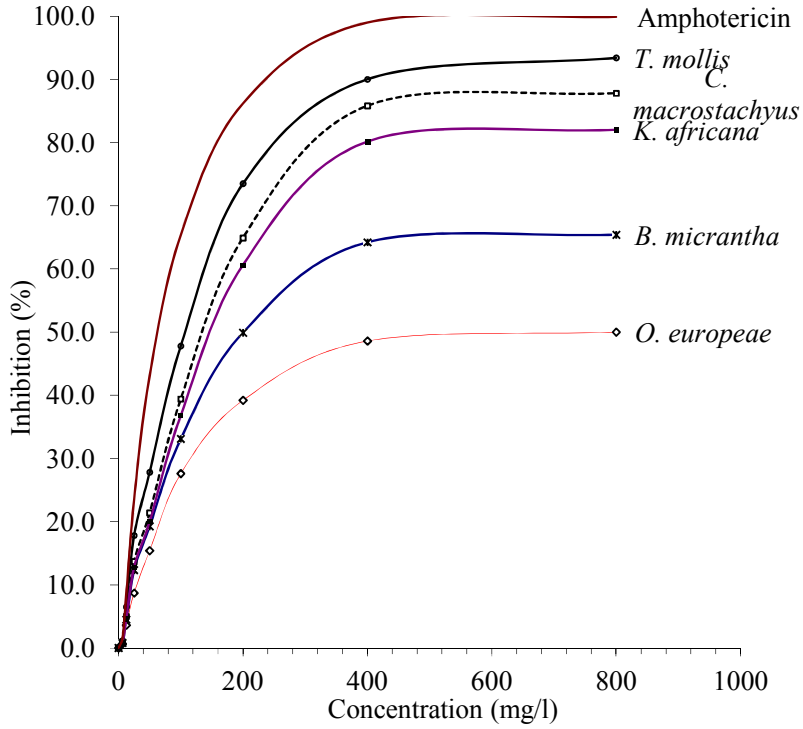

Fig. 1. DPPH radical scavenging activity of five plant extracts. Data are presented as mean ± SD, n=3 experiments, *p* values; *: p < 0.05, **: p < 0.01, ***: p < 0.001

Table 1. The phytochemical components of the plant extracts based on the preliminary extract screening

Phytochemical compound	*Olea europa*	*K. africana*	*Terminalia mollis*	*Croton macrostachyus*	*Bridella micrantha*
Tannins	++	++	+++	++	++
Phenols	-	-	+++	+	+++
Flavonoids	+	++	++	++	++
Steroids	-	+	+	+	+++
Alkaloids	+	++	-	+++	-
Saponins	+	++	+++	++	-
Alkaloid	++	-	++	-	+
Phlobatannin	-	+	+	-	-
Anthraquinone	+	++	+++	-	+
Cardiac glycoside	++	++	++	-	+
Terpenoids	-	+++	+	-	++
Polyphenols	++	-	-	-	+++
Cumarins	+	++	-	-	+++
Anthocyanins	+	-	++	++	-
Glycosides	-	+++	-	+	-
Triterpenoids	-	+	+	++	

+++ = high amount; ++ = moderate amount; + = trace amount; - = Not detected

Table 2. Optimal efficacy, LC_{50} and LC_{90} of test drugs against promastigote form of the parasites for 24 h period

	Test drugs						Parameter and statistics	
Concentration (μg/ml)	**Amphotericin**	***T. mollis***	***C. macrostachyus***	***K. africana***	***B. micrantha***	***O. europaea***	**F-value**	**P-value**
IC_{90}	-	-	460		242.6	150.4	89.221	p <0.01
IC_{50}	122.0	175.3	110.5		76.5	57.6	112.489	p <0.01

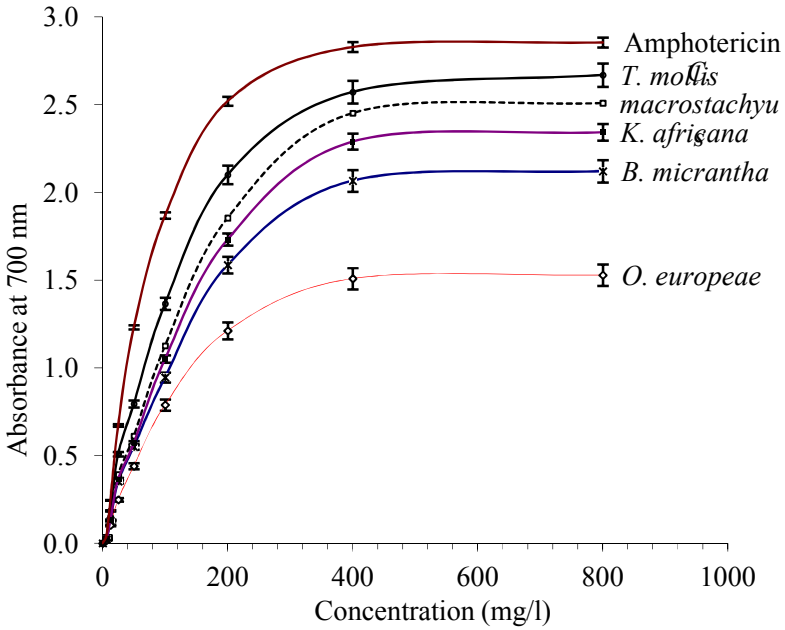

Fig. 2. Reducing power of extracts from five plant extracts. Data are presented as mean ± SD, n = 3 experiments, p values; *: p < 0.05, **: p < 0.01, ***: p < 0.001

which was also dose-dependent, the observed results were in agreement with previously reported [56,57,58]. The found results could be explain the important ability of our extracts to scavenging free radical such as ROS, inhibiting lipid peroxidation, avoiding DNA damage and prevent carcinogenesis processes [59]. This strong antioxidant activity of *T. mollis*, *C. macrostachyus* and *K. africana* may be due to the affluence of secondary metabolites such as alkaloides, flavonoids and polyphenols which was confirmed with our study of the phytochemical compounds (Table 1).

4. CONCLUSIONS

The aim of this study was to test whether different plant extracts used for traditional medicine practices could be promising sources of natural antioxidants. The robust antioxidant capacity determined by the DPPH assay and FRAP assay suggest that phenolic and flavonoid contents in the plants are useful indicators of antioxidant properties. The knowledge of traditional medicine practices can be a source of useful information for the isolation of natural extracts to develop new products for natural health care and well-being of domestic animals. Further investigations for potential applications of new natural antioxidants require anyway, elucidation of the chemical composition of phenolic and flavonoid in vivo studies in order to better establish the functionality of the examined plant species against a wide range of tropical diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was done in accordance with ethical guidelines of Maseno University. The approvals were further obtained from the Scientific and Ethics Review Unit (SERU) (PROTOCOL NO. KEMRI/SERU/CBRD/205/3968). The research permit was granted by the National Commission for Science, Technology and Innovation (License No: NACOSTI/P/21/8451).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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