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Comparative Anti-oxidant Activity of Aqueous and Organic Extracts from Kenyan *Ruellia lineari-bracteolata* and *Ruellia bignoniiflora*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors COW and KC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JAO, FWM, PGK and JK managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants play a significant role in treatment and prevention of many diseases in humans worldwide. *Ruellia* species belong to the family *Acanthaceae* and have been used widely for medicinal purposes. The objective of this study was to evaluate the comparative *In vitro* anti-oxidant activity of two Kenyan *Ruellia* species viz. *Ruellia lineari-bracteolata* (RLB) and *Ruellia bignoniiflora* (RBK). The plant materials were extracted with aqueous, ethyl acetate and methanol. The extracts were subjected to phytochemical screening according to standard procedures and

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anti-oxidant activity determined using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Comparative anti-oxidant activity for methanolic, ethyl acetate and aqueous extracts of RLB and RBK exhibited IC_{50} values of (2.7, 29.3, 7.2 and 24.4, 237.2, 66.4 µg/ml) respectively. Among the three extracts, methanolic extract showed better activity (2.7 µg/ml) comparable to ascorbic standard (2.1 µg/ml). Between the two *Ruellia* species, RLB showed a significant difference ($p<0.05$) in anti-oxidant activity as compared to RBK extracts. Phytochemical screening showed the presence of terpenoids, saponins, flavonoids, tannins and glycosides. Flavonoids and tannins are the main phytoconstituents responsible for anti-oxidant activity. In conclusion, the potent anti-oxidant activity of these plants makes them useful in development of medicinal drugs for treatment and prevention of degenerative diseases.

Keywords: Ruellia lineari-bracteolata; Ruellia bignoniiflora; extracts; DPPH; phytochemicals; anti-oxidant.

ABBREVIATIONS

UoN : University of Nairobi
DPPH : 2, 2-Diphenyl-1-picrylhydrazyl
IC_{50} : Inhibitory concentration at 50%
JKUAT : Jomo Kenyatta University of Agriculture and Technology

1. INTRODUCTION

Since ancient times, medicinal plants have played a significant role in treatment and prevention of various diseases [1]. In spite of modern systems of drug discovery and development, traditional knowledge systems have led to the discovery of valuable drugs [2]. Herbal medicine nowadays forms an integral part of alternative medicines for both human and livestock. In comparison with conventional drugs, traditional medicines are cheaper, easy to consume and are locally available [3]. Several herbal plants possess some compounds which have anti-oxidant properties that protect the cell against the damaging effects of reactive oxygen. Reactive oxygen species play a critical role in the pathophysiology of degenerative diseases such as cancer, cardiovascular diseases, arthritis, Alzheimer's disease and Parkinson's disease [4,5]. Despite the fact that our bodies are being protected by the natural anti-oxidant defense mechanisms, there is always demand for anti-oxidants from natural sources [6]. Secondary metabolites from medicinal plants such as flavonoids and phenolic compounds possess strong anti-oxidant activity which could help in protecting the cells against free radicals that cause oxidative damage [7]. These compounds from herbal plant materials terminate the action of free radicals thereby protecting the body from various diseases [8]. However due to emergence of various diseases worldwide, there is a growing need for scientists to discover the untapped reservoir of herbal medicinal plants. *Ruellia* is a genus of a flowering plants commonly known as wild petunias [9]. Most of the plant species of *Ruellia* has been widely used as anti-diabetic, antipyretic, gastroprotective, antimicrobial, analgesic, anti-oxidant and anticancer against the epidermis of nasopharynx region [10]. Hence, the present study aimed at comparing the phytochemical constituents and anti-oxidant activity of Kenyan RLB and RBK.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plants

Plant samples of RLB and RBK were collected from Isiolo and Makueni county Kenya respectively. The plants were taxonomised and Voucher specimens (Ref. nos. UoN/2015/003 of July 3, 2015 and 2013/811 of March 15, 2013 respectively) deposited at the Department of Botany Herbarium, University of Nairobi. The collected plant materials were dried in shade at ambient temperature, ground and milled to coarse powder by use of an electrical grinder made from Mechanical Engineering Department of Jomo Kenyatta University of Agriculture and Technology.

2.2 Aqueous Extraction

The crude aqueous extracts of both RLB and RBK were prepared according the techniques described by [11]. 50 g of plant powder was mixed with 500 ml of distilled water in 1L flask and boiled. The residues were filtered using Whatman No.1 filter paper. The extracts were then evaporated to dryness by freeze dryer (Christ Alpha 1-4 LD) and stored at 4℃ until required for phytochemical screening and anti-oxidant activity.

2.3 Organic Extraction

50 g of plant powder of *Ruellia* species were prepared by soaking in 500 ml of Methanol and ethyl acetate respectively, for 72 hours. The extracts were concentrated using a rotary evaporator (BUCHI R-200) at 45℃ and stored at 4℃ until used.

2.4 Phytochemical Screening

Phytochemical test of methanol, aqueous and ethyl-acetate extracts were carried out using standard procedures by [12].

2.5 Quantitative Analysis

2.5.1 Phenolic content

Dried (finely ground) plant material (200 mg) was put in a 50 ml glass beaker. Ten ml of aqueous acetone (70%) was added and extracted while shaking for 20 min at room temperature. The contents of the beaker were then transferred to centrifuge tubes and subjected to centrifugation for 10 min at 3000 g (Centurion 6000 Series). The supernatant was collected and kept on ice and then analysed for total phenols. Folin-Ciocalteu method described by [13] was used for the determination of total phenols in the supernatant. Tannic acid was used as the standard to prepare working standards. To the supernatant, 0.3 ml of it was taken in a test tube and 2.2 ml of distilled water added followed by 1.25 ml of Folin-Ciocalteu reagent and then 6.25 ml of sodium carbonate solution. The tube was vortex and absorbance recorded at 725 nm after 40 min in a UV spectrophotometer (UV-1800 Shimadzu). The amount of total phenols was calculated as tannic acid equivalent from the calibration curve and recorded.

2.5.2 Flavonoid content

The total flavonoid concentration was measured by the aluminium chloride colorimetric assay [14]. The extract (0.1 g) was added to a 20 ml volumetric flask containing 4 ml of double distilled water. To the above mixture, 0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. After 6 minutes, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with double distilled water. The solution was mixed well and the absorbance was measured at 510 nm against a blank. The flavonoid content was determined using catechin as standard.

2.6 *In-vitro* Anti-oxidant Activity

DPPH scavenging activity was carried out according to the procedure described by [15] with slight modification. The method is based on reduction of DPPH radicals from dark blue in color to yellow colored solution. DPPH solution was prepared by adding 3.94 mg (DPPH) in 100 mL methanol. Concentrations ranging from 3.9-500 µg/ml for both extracts and ascorbic acid were prepared. After 30 minutes of incubation in a dark room, absorbances were read at 517 nm. The scavenging inhibitory effect of DPPH was calculated according to the following formula;

Percentage (%) Inhibition = (Absorbance $\underline{\text{of}}_{DPPH}$- absorbance of $\underline{}_{SAMPLE}$)/ Absorbance $_{DPPH}$) × 100

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening of Plant Materials

Comparative phytochemical screening results of aqueous, ethyl acetate and methanolic extracts of RLB and RBK aerial parts are presented in Table 1.

Knowledge of phytochemical composition of plants is important in discovery of therapeutic agents for treatment and prevention of various diseases. As described by [16], the most important bioactive plant constituents include saponins, alkaloids, tannins, flavonoids and phenolic compounds. The present study on phytochemical screening of aqueous and organic extracts from both RLB and RBK showed the presence of saponins, phenols, flavonoids, glycosides and terpenoids. Whereas all the extracts showed absence of alkaloids, ethyl acetate extract showed absence of saponins and phenols for both plants.

3.2 Quantitative Analysis Results

The flavonoid and phenolic content in the samples was expressed in mg/ml and presented in Table 2.

Flavonoids content was found to be highest in RLB species with a concentration of 17 mg/ml followed by RBK with 15.1 mg/ml. However, RLB and RBK showed a less concentration of phenolic content with 1.2 and 1.6 mg/ml respectively. This is attributed to the nature of phenolic compounds in form of dried ground

Table 1. Phytochemical screening results

Phytochemical	Plant sample	Type of solvents		
		Methanol	Aqueous	Ethyl acetate
Saponins	RLB	+	+	-
	RBK	+	+	-
Phenolics	RLB	+	+	-
	RBK	+	+	-
Alkaloids	RLB	-	-	-
	RBK	-	-	-
Glycosides	RLB	+	+	+
	RBK	+	+	+
Flavonoids	RLB	+	+	+
	RBK	+	+	+
Terpenoids	RLB	+	+	+
	RBK	+	+	+

Key: '-' Absent, '+' Present

powder which makes them difficult to extract due to their 'fixing' in situ [12]. As described by [17], phenols and flavonoids are the main groups of compounds that act as primary free anti-oxidant scavenging radicals. The phenolic and flavonoids compounds in medicinal plants act as anti-oxidants due to their redox potential thus allowing them to act as reducing agents or free radical quenchers [18].

3.3 *In-vitro* Anti-oxidant Activity

Different concentrations of aqueous and organic extracts ranging from (3.9-500 µg/ml) were tested for their anti-oxidant activity and results presented in the Figs. 1-3.

Table 2. Showing the total flavonoids and phenolic content in RLB and RBK species

Samples	Phytochemical content (mg/ml)	
	Flavonoid	Phenolic
RBK	15.1± 0.81	1.2± 0.59
RLB	17.6±0.41	1.6± 0.10

From the results presented in Figs. 1-3, it was observed that methanolic extract exerted higher anti-oxidant activity as compared to aqueous and ethyl acetate extracts and its anti-oxidant activity was comparable to Ascorbic acid. The anti-oxidant activity increased with increase in extract concentration. This shows the capacity of these plants to donate electrons to neutralize free radicals and form a stable product [19].

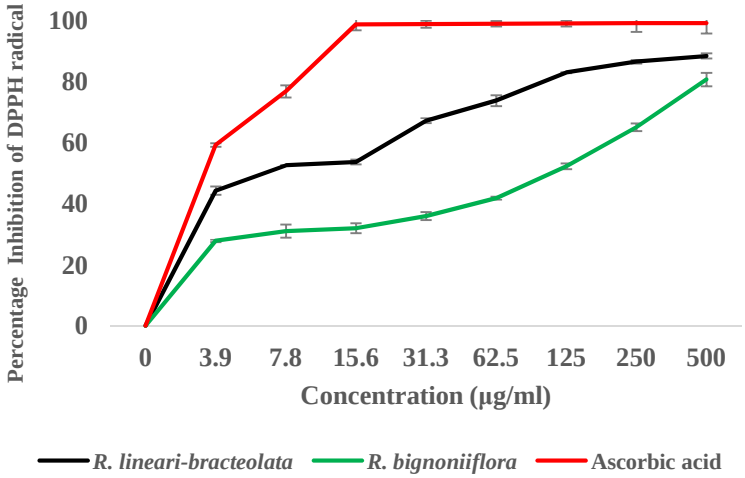

Fig. 1. Results showing comparative anti-oxidant activity of aqueous extracts of RLB and RBK

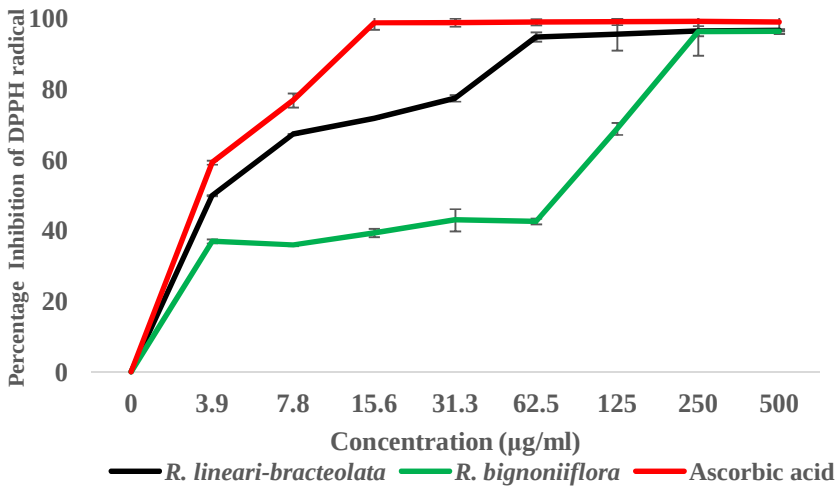

Fig. 2. Results showing comparative anti-oxidant activity of Methanolic extracts of RLB and RBK

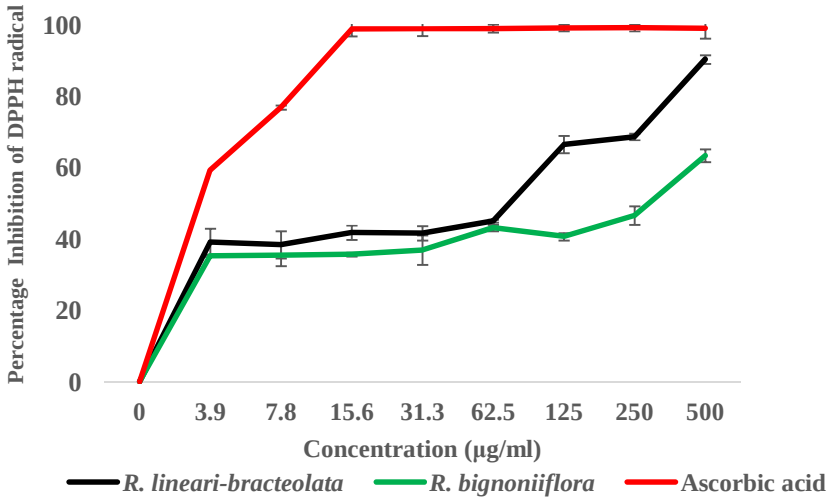

Fig. 3. Results showing comparative anti-oxidant activity of ethyl acetate extracts of RLB and RBK

3.4 Comparative Determination of 50% Inhibitory Concentration (IC_{50})

The concentration required to inhibit 50% of DPPH radical (IC_{50}) was determined using the regression line of probit according to the $\log_{10}$ of the extract concentration for aqueous, methanolic and ethyl acetate extracts of RLB and RBK species and results presented in Table 2.

It was seen that RLB methanol extract had a stronger effect of DPPH scavenging free radical with an average IC_{50} value of 2.7 µg/ml comparable to ascorbic acid as a reference standard with IC_{50} value of 2.1 µg/ml. The higher activity of methanol extract could be attributed to the polarity of solvent since phenols and flavonoids are highly soluble in polar solvents [20]. The high concentration of the compounds in the extracts in the polar solvent may have led to the increased activity. Anti-oxidant activity for methanol, ethyl acetate and aqueous extracts of both (RLB and RBK) exhibited IC_{50} values of 2.7, 29.3, 7.2 and 24.4, 237.2, 66.4 µg/ml

respectively. This shows that the plants had a dose-dependent anti-oxidant activity on the DPPH radical. RBK showed a considerably less hydrogen donating ability with IC_{50} value of 237.2 µg/ml compared with other plant extracts. Between the two *Ruellia* species, RLB showed a significant ($p<0.05$) anti-oxidant activity as compared to RBK extracts.

Table 3. IC_{50} values in (µg/ml)

Name of extract	Sample	IC_{50} values (µg/ml)
Aqueous extract	RLB	7.2
	RBK	66.4
Methanolic	RLB	2.7
extract	RBK	24.4
Ethyl-acetate	RLB	29.3
extract	RBK	237.2
Standard	Ascorbic acid	2.1

4. CONCLUSION

The results of anti-oxidant activity in the present study indicated that RLB showed a better anti-oxidant activity compared to RBK plant. The results also clearly indicated that methanolic, ethyl acetate and aqueous extracts of both plants showed a good anti-oxidant activity. Among the three extracts, methanol extract showed better activity comparable to ascorbic standard. The anti-oxidant activity of both RLB and RBK is reported for the first time in this study and may be attributed to the presence of flavonoids and phenolic compounds. The strong anti-oxidant activity of RLB, comparable to standard ascorbic acid, could provide an alternative promising source of natural anti-oxidants for medicinal and commercial uses.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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