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Comparative Preliminary Phytochemical and Biological Investigations on *Encephalartos kisambo* Faden & Beentje and *Encephalartos ferox* G. Bertol. Cultivated in Egypt

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ABSTRACT

Plants are important sources of bioactive principles. This study aimed to compare the phytochemical and biological profiles of *Encephalartos kisambo* Faden & Beentje (*E. kisambo*) and *Encephalartos ferox* G. Bertol (*E. ferox*) leaves (family Zamiaceae) for the first time. The phytochemical qualitative investigation of the total methanolic extract (TME) revealed that both *E. kisambo* and *E. ferox* contain flavonoids, tannins, phenolics, sterols or triterpenes, carbohydrates and saponins. However, anthraquinone glycosides, cardiac glycosides, and alkaloids were absent in both plants. The total phenolic content (TPC) and total flavonoid content (TFC) of both plants were measured. The TPC of *E. kisambo* (62.08 µg GA E/mg) is 1.4-fold that of *E. ferox* (45.93 µg GA E/mg). However, the TFC in *E. kisambo* (24.68 µg R E/mg) is comparable to that of *E. ferox* (25.76 µg R E/mg). Biological profile, including antioxidant and anti-inflammatory activities was investigated for the TME and their four fractions namely petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH). The antioxidant potential was evaluated using the DPPH and ABTS radical scavenging techniques. For both DPPH and ABTS, the TME of *E. kisambo* exhibited antioxidant activity stronger than *E. ferox*. Amongst the investigated fractions, the EtOAc and *n*-BuOH of *E. kisambo* showed the highest antioxidant effect. The DPPH assay values of the EtOAc and *n*-BuOH fractions are 7.572 µg/mL ± 0.105 and 10.25 µg/mL ± 0.161, respectively. The ABTS assay results for the same fractions are 3.145 µg/mL ± 0.15 and 5.551 µg/mL ± 0.43, respectively. The anti-inflammatory activity was tested *in vitro* by measuring nitric oxide (NO) % inhibition using lipopolysaccharide (LPS) treated RAW 264.7 macrophages derived from mice. The TME of *E. ferox* exhibited a higher anti-inflammatory potential than *E. kisambo*. The effect of 10 µg/mL of the EtOAc fractions of *E. kisambo* and *E. ferox* was approximately 0.69-fold and 0.66-fold that of the positive control (1 mM L-NAME), respectively. These promising antioxidant and anti-inflammatory activities of both plants warrant further future studies for isolation and identification of the active components.

Keywords: Phytochemical profile, *Encephalartos*, DPPH, total phenolic content, anti-inflammatory, total flavonoid content, ABTS.

1. INTRODUCTION

The search for natural medications is undergoing a significant surge due to the unfavourable side effects and high prices of synthetic medications.[1,2] Natural occurring products have long been a focal point of scientific research, having been used as therapeutic agents for disease treatment since ancient times.[3] According to the World Health Organization, over 80% of the global population relies primarily or partially on traditional including herbal medicine for primary healthcare.[4] Oxidative stress and inflammation are key drivers of numerous chronic diseases, including neurodegenerative conditions, cardiovascular abnormalities, cancer, diabetes and

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autoimmune pathologies.[5–7] Various plant secondary metabolites like alkaloids, tannins, flavonoids, phenolics, terpenoids, and glycosides have demonstrated significant *in vitro* antioxidant and anti-inflammatory activities.[8] Medicinal plants represent a rich source of natural antioxidants that assist in scavenging free radicals, thereby alleviating oxidative stress and protecting cells from damage.[9] Moreover, many plant-derived compounds exhibit strong anti-inflammatory effects through the regulation of critical inflammatory pathways.[10,11] Gymnosperms are a group of seed-producing plants that include some of the oldest living plant lineages, such as the order Cycadales, which is recognized for its richness in biologically active compounds.[12] This order is divided into two families: Cycadaceae and Zamiaceae.[13] Among family Zamiaceae, the genus *Encephalartos,* the second most extensive genus within the Zamiaceae family with 68 currently recognized taxa,[14] is notable for its limited geographic distribution in Africa and its critical conservation status.[15] Many species within the genus *Encephalartos* are critically endangered due to habitat loss, illegal collection, and extremely slow growth rates, placing them among the most threatened plant taxa globally.[16] *Encephalartos* possess stout trunks and crown-like rosettes of compound pinnate leaves with spiny, thick cuticled leaflets adapted to arid conditions. They are dioecious cycads with beetle pollinated cones and animal assisted seed dispersal.[17–20] *E. kisambo* and *E. ferox*, family Zamiaceae, were not investigated previously for their phytochemistry. The published literature for the biological screening is scarce. That motivated us to carry out this study on the leaves of *E. kisambo* and *E. ferox*, which were screened for their phytochemical profile including the TPC and TFC. In addition, the antioxidant and anti-inflammatory potentials were assessed *in vitro*.

2. METHODS

2.1. Plant Material

Leaves of *E. kisambo* and *E. ferox.* were obtained from El Abd garden, positioned 68 km along the road of Cairo-Alexandria, Egypt, in March 2021. The two plants were kindly provided by Mr Rabea Sharawy (general consultant at Alarma gardens) and were identified by Dr. Esraa Ammar, Department of Botany (Plant Ecology), Faculty of Science, Tanta University. A voucher specimen No. PGG-014 (*E. kisambo*) and No. PGG-015 (*E. ferox*) were placed in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Tanta University, Egypt. The plant materials were dried at room temperature in the shade, pulverized to get a fine powder, and stored in tightly sealed amber colored containers in refrigerator until further use.

2.2. Chemicals and Reagents

Methanol (MeOH), petroleum ether (PE) (60-80°C), dichloromethane (DCM), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) were utilized for extraction and fractionation. All used solvents were of analytical grade and purchased from Iso-Chem Pharmaceutical Chemical Co., Egypt. α-Naphthol (Sigma Chemical Co. St., Louis, USA) was utilized in the preparation of Molisch's reagent, Mayer's and Dragendorff's reagents were prepared in the laboratories of the Department of Pharmacognosy, Faculty of Pharmacy, Tanta University. Sulphuric acid, glacial acetic acid, 10 % ferric chloride (Iso-Chem Pharmaceutical Chemical Co., Egypt) and sodium hydroxide (Inter. Trade Co., Egypt) were used in the preliminary phytochemical screening. The total phenolic content (TPC) assay was performed using Folin–Ciocalteu reagent, gallic acid and sodium carbonate (Sigma Chemical Co. St., Louis, USA). The total flavonoid content (TFC) was assessed using aluminum chloride, sodium acetate and rutin (Sigma Chemical Co. St., Louis, USA).

2.3. Material for Biological Activity

2.3.1. *Plant Materials*

The prepared TMEs and their fractions for both plants were separately dissolved in dimethyl sulfoxide (DMSO, 0.1% v/v) for anti-inflammatory activity and in MeOH for antioxidant activity.

2.3.2 *Materials for Antioxidant Studies*

Antioxidant potential was investigated using two different assays (DPPH and ABTS) and Trolox was used as a standard reference (Sigma Chemical Co., St. Louis, MO.). For DPPH (2,2-Diphenyl-1-picrylhydrazyl), 0.1% solution in MeOH was used. For ABTS assay, the materials used were 2,2`-azino-bis-(3-ethyl benzthiazoline-6-sulfonic acid) (ABTS), MeOH, distilled water and potassium persulphate.

2.3.3. *Materials for Anti-inflammatory Studies*

RAW 264.7 cells: mouse macrophage cell line from Nawah Scientific Inc. (Mokatam, Cairo, Egypt), Dulbecco's Modified Eagle's Medium (DMEM) (Corning, USA), fetal bovine serum (10%), penicillin at a concentration of 100 U/mL, streptomycin sulphate at a concentration of 100 µg/mL and L-glutamine (2 mM), phosphate buffered saline, LPS, DMSO, Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME, 1 mM) and Griess reagent.

2.4. Extraction and Fractionation

The powdered *E. kisambo* and *E. ferox* leaves (5 Kg each) were separately extracted by 95% MeOH (3 × 10 L) using cold maceration until complete extraction. The solvent was evaporated at 40 °C under reduced pressure, yielding crude TMEs of 525 g (10.5 %) and 492 g (9.84 %) for *E. kisambo* and *E. ferox*, respectively. The crude TMEs of *E. kisambo* (280 g) and *E. ferox* (250 g) leaves were separately dissolved in MeOH and mixed with distilled water (1:1), then sequentially fractionated by successive extraction with PE (60–80 °C), DCM, EtOAc and *n*-BuOH to yield 28.5 g (10.18

%), 16.3 g (5.83 %), 19.4 g (6.93 %) and 18.5 g (6.61 %), respectively for *E. kisambo* and 19.7 g (7.88 %), 17.8 g (7.12 %), 10.2 g (4.08 %) and 15.0 g (6 %), respectively for *E. ferox.*

2.5. Chemical Tests for Phytochemical Screening

Preliminary phytochemical analyses were conducted to detect the dominant classes of secondary metabolites. The following tests were employed: sodium hydroxide test (flavonoids), ferric chloride (tannins and phenolics), Salkowski test (sterols and triterpenoids), Molisch's test (carbohydrates), froth test (saponins), modified Borntrager's (anthraquinones), Keller-Killiani test (cardiac glycosides) and Mayer's test (alkaloids).[21–24]

2.6. Total Phenolic Content Assessment

The TPC was assessed using the Folin Ciocalteu method.[25] The TMEs of *E. kisambo* and *E. ferox* were reconstituted in MeOH at a concentration of 1 mg/mL. For the assay, 10 µL of either the sample or the gallic acid standard was mixed with 100 µL of diluted Folin–Ciocalteu reagent (1:10) in a 96-well microplate ($n = 3$). Subsequently, 80 µL of 1 M sodium carbonate solution was added, and the mixture was incubated at 25°C in the dark for 20 minutes. The absorbance of the blue colored complex formed was read at 630 nm. Results were represented as [mean ± standard deviation (SD)], and a standard gallic acid curve was utilized to determine the TPC. Absorbance measurements were obtained using a microplate reader (FluoStar Omega).

2.7. Total Flavonoid Content Measurement

The TFC was measured in a microplate (aluminum chloride method).[26] Samples, from TMEs, were prepared to have 4 mg/mL in MeOH. Sample/standard (15 µL) was aliquoted in a 96-well microplate ($n = 3$) and completed to 190 µL by MeOH followed by 30 µL of 1.25 % aluminum chloride solution and lastly added 30 µL of 0.125 M sodium acetate. The mixture was then incubated in the dark at 25 °C (5 minutes). The intensity of the developed yellowish coloration was measured at 420 nm. Data were expressed as mean ± SD. The rutin calibration curve was employed to quantify the TFC. Absorbance measurements were performed utilizing a FluoStar Omega microplate reader.

2.8. Biological Activity

2.8.1. *Antioxidant Activity*

DPPH assay

In accordance to a reported technique,[27] the free radical DPPH assay was executed. Briefly, an aliquot of 100 µL of freshly prepared DPPH (0.1% w/v in MeOH) was added to 100 µL of the test sample in a 96-well plate ($n = 6$) where 30 minutes in the dark incubation at room temperature was applied to the mixture. Upon completion of the incubation period, the color intensity was measured at 540 nm. Results are expressed as mean ± SD using the equation below:

$$\text{Percentage inhibition} = \frac{\text{Average absorbance of blank} - \text{average absorbance of the test}}{\text{Average absorbance of blank}} \times 100$$

Trolox standard curve was implemented as positive control. An aliquot (100 µg/mL) of the TMEs of *E. kisambo* and *E. ferox* for determination of the % inhibition of DPPH radical. For determination of the IC_{50} of each test fraction sample, different concentrations were prepared in MeOH. For *E. kisambo*, the concentrations of the EtOAc and *n*-BuOH fractions were 6.25, 12.5, 25.0, 50.0 and 100.0 µg/mL, while for the PE and DCM fractions were 12.5, 25.0, 50.0, 100 and 200.0 µg/mL. For *E. ferox,* the concentrations of the EtOAc and *n*-BuOH fractions were 12.5, 25.0, 50.0, 100.0 and 200.0 µg/mL, for the DCM fraction were 31.25, 62.5, 125.0, 250.0 and 500.0 µg/mL, and for the PE fraction were 125.0, 250.0, 500.0, 1000.0 and 2000.0 µg/mL. From a Trolox stock solution (20.0 µg/mL MeOH), five serial dilutions were prepared (12.5, 10.0, 7.5, 5.0 and 1.25 µg/mL).

ABTS assay

The assay was employed following a described method,[28] in microplate, incorporating minor modifications. To prepare the ABTS solution, 1 mL of ABTS stock (192 mg in 50 mL of distilled water) was mixed with 17 µL of potassium persulfate (140 mM). This mixture was then left in the dark for 24 hours to allow for radical generation. Afterward, 1 mL of the resulting solution was diluted to 50 mL with MeOH to create the working ABTS solution used in the assay. For the assay, 190 µL of the working ABTS reagent was combined with 10 µL of the test sample in one well of a 96-well microplate. This process was repeated six times ($n = 6$) for each sample. The reaction mixture was incubated for 30 minutes in the dark at 25°C. Following incubation, the reduction in ABTS color intensity was measured at a wavelength of 734 nm. Results are expressed as mean ± SD, calculated using the equation below:

$$\text{Percentage inhibition} = \frac{\text{Average absorbance of blank} - \text{average absorbance of the test}}{\text{Average absorbance of blank}} \times 100$$

A Trolox standard curve was employed as the positive control. A solution of 100 µg/mL was prepared for each MeOH extract of *E. kisambo* and *E. ferox* for determination of the % inhibition of ABTS radical. For determination of the IC_{50} of each test fraction sample, different concentrations were prepared in MeOH. For *E. kisambo,* the concentrations of the PE fraction were 6.25, 12.5, 25.0, 50.0 and 100.0 µg/mL, for the DCM fraction were 1.25, 2.5, 5.0 ,10.0 and 20.0 µg/mL, for the EtOAc fraction were 0.625, 1.25, 2.5, 5.0 and 10.0 µg/mL and for the *n*-BuOH fraction were 1.25, 2.5, 5.0, 7.5 and 10.0 µg/mL. For *E. ferox,* the concentrations of the PE fraction were 30.0, 60.0, 120.0, 160.0 and 200.0

µg/mL, for the DCM fraction were 3.125, 6.25, 12.5, 25.0 and 50.0 µg/mL, while for the EtOAc and *n*-BuOH fractions were 1.25, 2.5, 5.0, 10.0 and 20.0 µg/mL. Trolox was prepared at the following final concentrations 2.5, 5.0, 6.25, 7.5 and 8.75 µg/mL.

2.8.2. *Anti-Inflammatory Activity*

DMEM cultured RAW 264.7 macrophages, supplemented with: streptomycin (100 mg/mL), penicillin (100 U/mL) and heat-inactivated fetal bovine serum (10 %) was kept in a humidified incubator (at 37°C) with 5 % CO_2. A stock suspension of cultured cells (0.5×10^6 cells/mL) was placed in a 96-well microplate and incubated overnight to allow for adherence. On the following day, the experimental groups were treated as follows: Non-induced control wells received medium containing 0.1% (v/v) DMSO (the vehicle). Inflammation control wells were treated with LPS at a concentration of 100 ng/mL in culture medium containing 0.1% (v/v) DMSO. Test sample wells (in triplicates) were treated with TMEs or fractions at a concentration of 10 µg/mL. These were dissolved in DMSO and diluted in LPS-containing medium to maintain a final DMSO concentration of 0.1% (v/v). Positive control wells received L-NAME at a concentration of 1 mM, which is a known anti-inflammatory agent. After 24 hours of incubation, NO production was quantified using the Griess assay [29] in all wells. Equal volumes of Griess reagent and culture supernatant were mixed and allowed to incubate at room temperature for 10 minutes. The resulting chromogenic diazonium salt was then measured spectrophotometrically at a wavelength of 520 nm. The percentage of NO inhibition by the TMEs and their fractions was calculated relative to the LPS-stimulated inflammation group and normalized based on cell viability, which was assessed using the Alamar Blue™ reduction assay.[30,31]

2.9. Statistical Analysis

Data are displayed as mean ± standard deviation (SD). The values of IC_{50} were determined using the GraphPad Prism 6® (https://www.graphpad.com) via conversion of concentrations to corresponding logarithmic values and applying a nonlinear-regression model [log(inhibitor) versus normalized response with variable slope]. All data are given as mean ± SD for triplicates ($n = 3$). Statistical significance between groups was evaluated using one-way ANOVA followed by Dunnett's post hoc test or Student's t-test, where appropriate. Statistical significance was considered at $p < 0.05$.

3. RESULT AND DISCUSSION

3.1. Screening of Phytochemical Content

The phytochemical screening results of *E. kisambo* and *E. ferox* leaves are listed in **Table 1**.

Table 1. Phytochemical preliminary screening results of *E. kisambo* and *E. ferox* leaves.

Test for \ Plant	*E. kisambo*	*E. ferox*
Flavonoids	+ve	+ve
Tannins and phenolic compounds	+ve	+ve
Sterols and/or triterpenes	+ve	+ve
Carbohydrates	+ve	+ve
Saponins	+ve	+ve
Anthraquinone glycosides	-ve	-ve
Cardiac glycosides	-ve	-ve
Alkaloids	-ve	-ve

-ve: negative, +ve: positive

According to the results, both *E. kisambo* and *E. ferox* contain flavonoids, tannins, phenolics, sterols or triterpenes, carbohydrates and saponins. However, anthraquinone glycosides, cardiac glycosides and alkaloids were absent in both plants. The phytochemical screening of *E. kisambo* and *E. ferox* is somewhat similar to many Cycadales, for example that of *Cycas rumphii* Miq. and *Zamia floridana* A. DC.[32] These plants share the presence of flavonoids, tannins, phenolics, sterols or triterpenes and carbohydrates while devoid of anthraquinone glycosides, cardiac glycosides, and alkaloids. Concerning saponins, they were detected in both *E. kisambo* and *E. ferox*, while neither *C. rumphii* Miq. nor *Z. floridana* A. DC. exhibited any presence of these compounds.

3.2. TPC and TFC

The results are listed in **Table 2** and **Figure 1**. Phenolic and flavonoid compounds play a crucial role as natural antioxidants by scavenging free radicals and protecting cells from oxidative stress.[33] Moreover, phenolics and flavonoids help suppress inflammation by modulating the production of pro-inflammatory mediators.[34] Due to their significant biological activity, the TPC and TFC in the selected plants were quantified to better understand their potential bioactivity. The results show differences in the amounts of phenolic and flavonoid compounds between the two *Encephalartos* species. *E. kisambo* contains a higher level of phenolics, while *E. ferox* has a slightly higher flavonoid content. These variations may influence their overall antioxidant and anti-inflammatory potential. Such differences could be linked to species-specific traits. TPC of *E. kisambo* was measured at 62.08 µg GA E/mg, which is 1.4 times higher than that of *E. ferox* (45.93 µg GA E/mg). While the TFC in *E. kisambo*, of 24.68 µg R E/mg, was comparable to that of *E. ferox*, which contained 25.76 µg R E/mg.

Table 2. TPC and TFC of the TMEs of *E. kisambo* and *E. ferox* leaves.

Sample	TPC (µg GA E/mg extract) ± SD	TFC (µg R E/mg extract) ± SD
E. kisambo	62.08 ± 2.70	24.68 ± 1.5
E. ferox	45.93 ± 2.68	25.76 ± 0.59

TPC: total phenolic content; TFC: total flavonoid content.

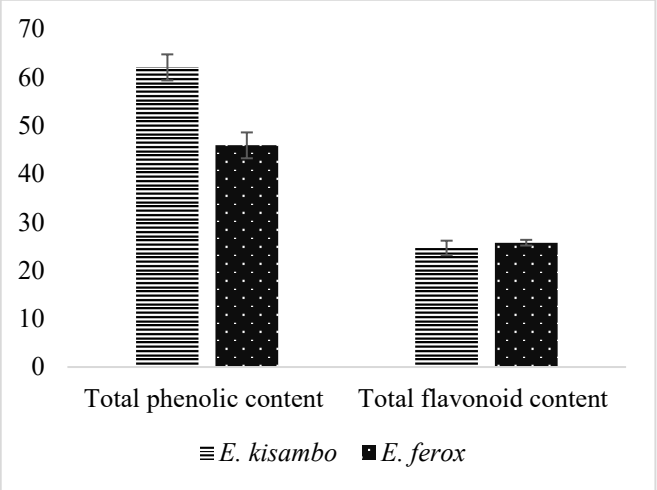

Figure 1. TPC and TFC of the TMEs of *E. kisambo* and *E. ferox* leaves (n = 3).

3.3. Biological Activity

3.3.1. *Antioxidant*

Antioxidant activities of TMEs of *E. kisambo* and *E. ferox* represented by measuring the % inhibition of the radicals of DPPH and ABTS which are illustrated in **Table 3**.

Table 3. % Inhibition of the DPPH and ABTS radicals by the TMEs of *E. kisambo* and *E. ferox.*

Sample	% Inhibition DPPH radical ± SD	% Inhibition ABTS radical ± SD
E. kisambo TME	44.91 % ± 1.52	72.07 % ± 0.49
E. ferox TME	37.60 % ± 1.08	61.26 % ± 1.43

TME: total methanolic extract

The DPPH radical scavenging capacity and results of the ABTS assay are listed in **Table 4** and shown in **Figures 2** and **3**. From the results, *E. kisambo* is more potent than *E. ferox* regarding the two methods of assay of antioxidant activity (DPPH and ABTS). For *E. kisambo,* the EtOAc fraction demonstrated the highest % for inhibiting the radicals of DPPH and ABTS, surpassing even the positive control (Trolox) in ABTS radical scavenging activity. For *E. ferox,* the *n*-BuOH fraction exhibited the highest % inhibition of DPPH and ABTS. Therefore, both plants hold potential as antioxidant remedies.

Given that *E. kisambo* exhibits a higher phenolic content compared to *E. ferox*, along with enhanced antioxidant activity, it suggests a significant role for the concentration of phenolic compounds in boosting antioxidant properties.

Table 4. DPPH and ABTS radical scavenging capacity of *E. kisambo* and *E. ferox* by different fractions.

Sample	DPPH IC_{50} ± SD (µg/mL)	ABTS IC_{50} ± SD (µg/mL)
E. kisambo		
PE	171.4 ± 13.72	42.22 ± 3.91
DCM	68.95 ± 2.41	10.70 ± 0.50
EtOAc	7.572 ± 0.105	3.145 ± 0.15
n-BuOH	10.25 ± 0.161	5.551 ± 0.43
E. ferox		
PE	278.4 ± 11.76	115.9 ± 10.65
DCM	77.37 ± 2.12	15.60 ± 0.91
EtOAc	28.40 ± 0.58	8.786 ± 0.76
n-BuOH	19.17 ± 0.43	8.333 ± 0.67
Trolox	7.217 ± 0.309	5.58 ± 0.104

PE: petroleum ether; DCM: dichloromethane; EtOAc: ethyl acetate; *n*-BuOH: *n*-butanol

3.3.2. *Anti-inflammatory*

The results of anti-inflammatory potential of *E. kisambo* and *E. ferox* TMEs and different fractions are listed in **Table 5** and shown in **Figure 4**.

Table 5. Anti-inflammatory activity (NO % inhibition) of *E. kisambo* and *E. ferox* TMEs and different fractions.

Sample	NO % inhibition ± SD
E. kisambo	
TME	21.49 ± 2.031
PE	12.44 ± 1.000
DCM	15.70 ± 2.142
EtOAc	59.33 ± 2.055
n-BuOH	24.86 ± 1.195
E. ferox	
TME	27.85 ± 1.687
PE	46.35 ± 1.336
DCM	43.91 ± 1.572
EtOAc	56.84 ± 2.152
n-BuOH	50.68 ± 1.624
L-NAME (1 mM)	85.84 ± 1.459

TME: total methanolic extract; PE: petroleum ether; DCM: dichloromethane; EtOAc: ethyl acetate; *n*-BuOH: *n*-butanol; L-NAME: Nω-Nitro-L-arginine methyl ester hydrochloride

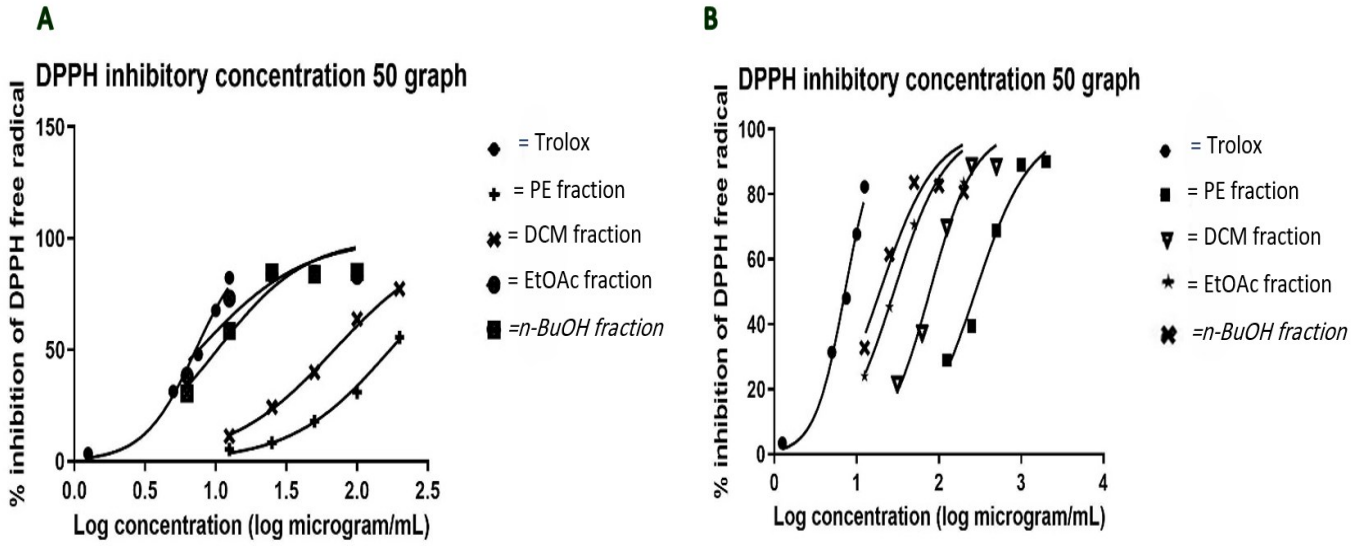

Figure 2. % Inhibition of DPPH radical by *E. kisambo* (A) and *E. ferox* (B) fractions (n = 6).

PE: petroleum ether; DCM: dichloromethane; EtOAc: ethyl acetate; *n*-BuOH: *n*-Butanol

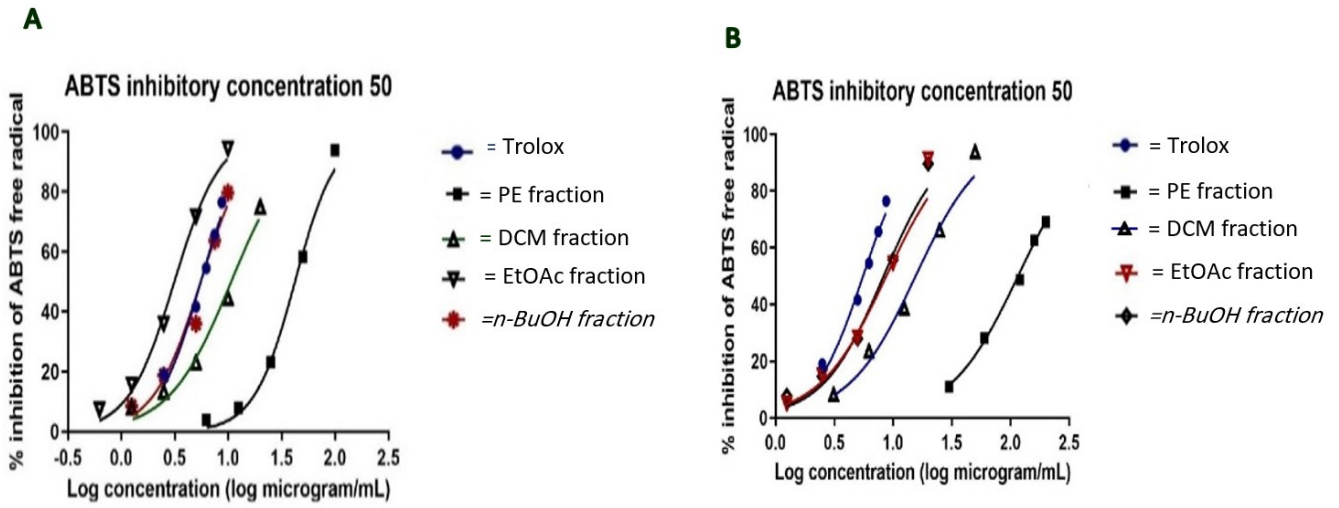

Figure 3. % Inhibition of ABTS radical by *E. kisambo* (A) and *E. ferox* (B) fractions (n = 6).

PE: petroleum ether; DCM: dichloromethane; EtOAc: ethyl acetate; *n*-BuOH: *n*-Butanol

TME of *E. ferox* exhibited a higher anti-inflammatory activity than *E. kisambo*. Amongst the tested fractions, the anti-inflammatory assay revealed that the EtOAc fractions of *E. kisambo* and *E. ferox* inhibited NO levels in LPS treated RAW 264.7 macrophages by (59.33 ± 2.055 and 56.84 ± 2.152, respectively) (**Table 5, Figure 4**). Based on these results the IC_{50} of the EtOAc extracts of both plants were determined at (10.29 and 12.16 µg/mL, respectively) (**Figure 5**). The EtOAc extracts of *E. kisambo* and *E. ferox* (10 µg/mL) exhibited 0.69-fold and 0.66-fold of positive control (1 mM L-NAME) which indicates that the plants are promising as anti-inflammatory.

Oxidative stress and inflammation are interlinked biological processes that contributes to the pathophysiology of multiple long-term diseases, including neurodegenerative abnormalities, cancer, diabetes and disorders of the cardiovascular system.[35] Natural antioxidants, particularly those derived from plant sources, play a vital role in neutralizing reactive oxygen species and enhancing cellular antioxidant capacity. Phenolic compounds, such as flavonoids and phenolic acids, have been extensively studied for their dual role as radical scavengers and modulators of inflammatory pathways.[36]

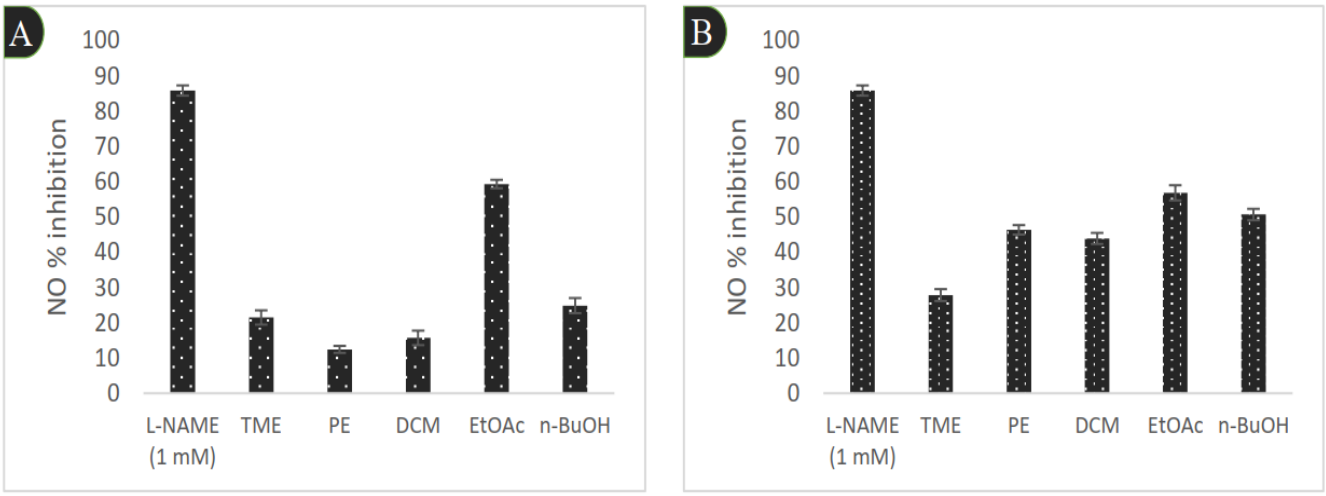

Figure 4. Anti-inflammatory activity (NO % inhibition) by TMEs and different fractions of *E. kisambo* (A) and *E. ferox* (B) (n = 3).

L-NAME: Nω-Nitro-L-arginine methyl ester hydrochloride; TME: total methanolic extract; PE: petroleum ether; DCM: dichloromethane; EtOAc: ethyl acetate; *n*-BuOH: *n*-Butanol

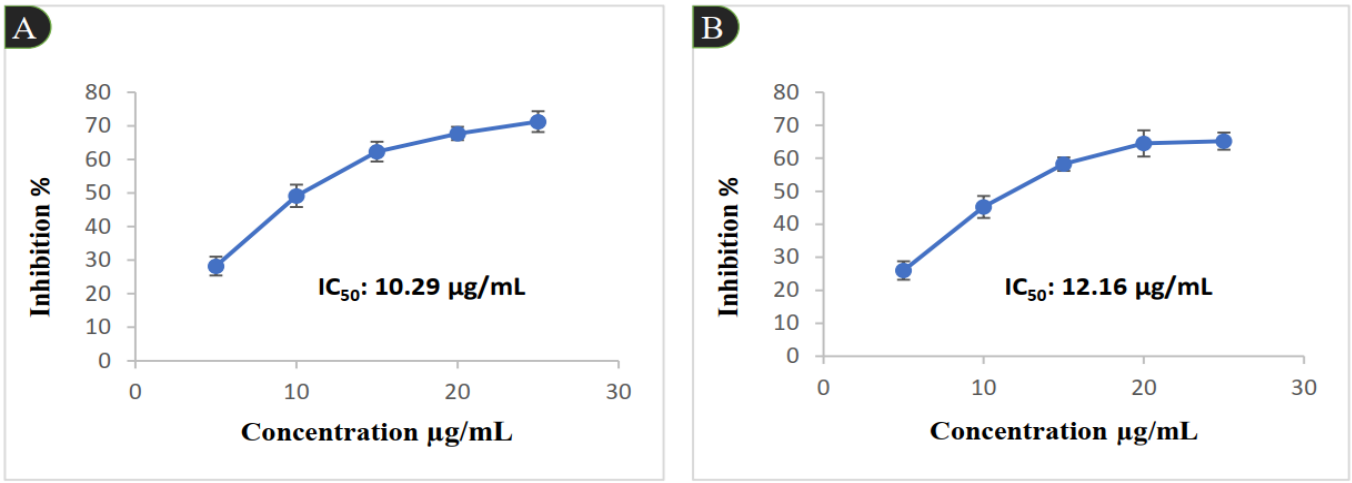

Figure 5. IC_{50} of NO % inhibition of the EtOAc fraction of *E. kisambo* (A) and *E. ferox* (B) (n = 3).

IC_{50}: half maximal inhibitory concentration

CONCLUSION

The phytochemical investigation of *E. kisambo* and *E. ferox* demonstrated the presence of several secondary metabolites like flavonoids, tannins, phenolics, sterols or triterpenes, carbohydrates and saponins. Conversely, both species were found to lack anthraquinone glycosides, cardiac glycosides and alkaloids. Quantitative analysis revealed that *E. kisambo* had a higher TPC than *E. ferox*, whereas a slightly similar flavonoid level was found in both species. In antioxidant assays, the TME of *E. kisambo* demonstrated higher antioxidant activity than that of *E. ferox* in both DPPH and ABTS assays. The EtOAc fraction of *E. kisambo* showed the strongest free radical scavenging capacity in both DPPH and ABTS assays, followed by its *n*-BuOH fraction. Moreover, the TME of *E. ferox* demonstrated higher anti-inflammatory potential than *E. kisambo*. The EtOAc fractions from both species markedly suppressed NO production in LPS treated RAW 264.7 macrophage cells, indicating notable anti-inflammatory activity.

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CONFLICT OF INTEREST

The authors have not declared any conflicts of interest.

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