The Journal of Phytopharmacology 2023; 12(4):211-217
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2023; 12(4): 211-217
July- August
Received: 06-06-2023
Accepted: 03-08-2023
Published: 31-08-2023

doi: 10.31254/phyto.2023.12401

Peter Maloba
Department of medical Laboratory Sciences, Masinde Muliro University of Science and Technology, P.O Box 190-50100, Kakamega, Kenya

Elias Mandela
Department of Pharmacognosy, Mount Kenya University, P.O Box 342-01000 Thika, Kenya

Sammy Kimoloi
Department of medical Laboratory Sciences, Masinde Muliro University of Science and Technology, P.O Box 190-50100, Kakamega, Kenya

Jared Onyancha
Department of Pharmacognosy, Mount Kenya University, P.O Box 342-01000 Thika, Kenya

Correspondence:
Dr. Jared Misonge Onyancha
Department of Pharmacognosy, Mount Kenya University, P.O Box 342-01000 Thika, Kenya
Email: jonyancha@mku.ac.ke

Antiinflammatory, Antinociceptive and Antioxidant activities of aqueous and methanolic stem bark extracts of *Fagaropsis hildebrandtii* (Engl.) Milne-Redh. (Rutaceae)

Peter Maloba, Elias Mandela, Sammy Kimoloi, Jared Onyancha

ABSTRACT

Background: *Fagaropsis hildebrandtii* has been widely used in African traditional medicine to treat various illnesses, particularly those associated with inflammation, pain and oxidative stress. However, experimental data on the anti-inflammatory, anti-nociceptive and antioxidant of the different parts of *F. hildebrandtii* is still limited. **Objectives:** The objectives of this study were to determine the phytochemical composition**,** anti-inflammatory, antinociceptive and antioxidant activities of stem bark extract of *F. hildebrandtii* from Makueni County, Kenya. **Materials and Methods**: The stem bark of *F. hildebrandtii* was extracted by maceration using water and methanol. Standard qualitative methods were then used to determine the presence or absence of various phytochemicals in the extracts. Total phenolic and flavonoid contents were determined by Folin-Ciocalteu and aluminium chloride calorimetric methods, respectively. Anti-inflammatory and anti-nociceptive activities of increasing water and methanol extracts doses (2-250mg/Kg) were tested using carrageenan-induced paw edema and acetic acid-induced writhing animal models, respectively in comparison with dexamethasone and aspirin as standard. Antioxidant activities of plant extracts were assayed by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method, with ascorbic acid as the standard. Data analysis was conducted by two-way ANOVA with appropriate post hoc tests. Statistical significance level was set at $p \leq 0.05$. **Results:** Phenols, flavonoids, alkaloids, saponins, tannins, terpenoids and coumarin were detected in the extracts. However, anthocyanins and anthraquinones were absent in both water and methanolic extracts. Notably, methanol extract had a high total phenolic level compared to water extract (34.590 ± 2.490 mgGAE/g vs. 7.893 ± 1.619 mgGAE/g; $p<0.05$). Total flavonoid content of *F. hildebrandtii* methanol extract was also higher in comparison to water extract (132.18 ± 0.26 mgCE/g vs. 42.68 ± 0.93mgCE/g; $p<0.05$). *F. hildebrandtii* water and methanol extracts had a radical scavenging activity IC_{50} of 0.98 µg/ml and 0.987 µg/ml respectively, which was significantly lower than 5.674 µg/ml for ascorbic acid, indicating a higher antioxidant potency. The percentage inhibition of carrageenan-induced hind paw edema by *F. hildebrandtii* extracts was not significantly different in comparison with 10 mg/Kg of dexamethsone standard over a 5-hour test period ($p>0.05$). *F. hildebrandtii* water and methanol extracts exhibited a dose-dependent inhibition of acetic acid-induced writhing in mice, reaching a maximum of 53.1 ± 6.1% and 80.24 ± 12.59%, respectively, at 250 mg/Kg. Notably, the percentage inhibition of acetic acid-induced writhing by 50 mg/kg and 250 mg/kg of *F. hildebrandtii* methanolic extract was similar to that of aspirin standard at 150 mg/kg (77.75 ± 19.64% vs. 80.24 ± 12.59% vs. 84.99 ± 3.26%, $p>0.05$, two-way ANOVA). **Conclusion:** *F. hildebrandtii* has anti-inflammatory, antinociceptive and antioxidant activities. These findings validate the use of *F. hildebrandtii* crude extracts among Kenyan ethnic groups in management of diseases associated with inflammation, pain, and oxidative stress.

Keywords: DPPH assay, Gallic acid, Paw edema, Phytochemisty and Traditional medicine.

INTRODUCTION

Communicable diseases including malaria, human immunodeficiency virus (HIV), tuberculosis (TB), schistosomiasis and leishmaniasis still affects a large number of people, particularly in developing countries [1]. These diseases are associated with immune response-mediated inflammation, pain and oxidative stress. Emerging infectious diseases like COVID-19 are also characterized by massive inflammation of the respiratory system as well as other multiple organ systems [2]. Moreover, non-communicable diseases (NCDs) including hypertension, diabetes mellitus, atherosclerosis, obesity, cancer, autoimmune diseases, depression, Parkinson's disease and arthritis, which are also on the rise, are characterized by chronic or low grade inflammation, pain and increased oxidative stress levels [3, 4]. Indeed, more than 50% of deaths worldwide can be attributed to inflammatory-related NCDs such as stroke, chronic kidney disease, ischemic heart disease, non-alcoholic fatty liver diseases and neurodegenerative diseases [5].

Currently, pharmacological management of inflammation rely on the use of corticosteroids, histamine-1 receptor inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs) and targeted monoclonal antibodies. However, these pharmacological agents do not provide optimal anti-inflammatory effects for all patients and are often unaffordable in most resource poor settings [6, 7]. Similarly, currently available analgesic and antioxidant drugs are sub-optimal in the management of various chronic pain and diseases associated with marked oxidative stress. This emphasizes the need for discovery, development, and validation of novel or alternative anti-inflammatory, analgesic, and antioxidant drugs.

Medicinal plants have remained an important resource for discovery of lead compounds and novel drugs with potent anti-inflammatory, analgesic, and antioxidant activities. These include salicylic acid from the Willows plant, morphine from the poppy opium and resveratrol from several plants [8-10]. Ethnobotanical studies indicate that plant parts of the genus *Fagaropsis* are widely used to treat various illnesses by various communities in the East African region [11]. In Kenya, *F. hildebrandtii* is used by the Kamba community to treat chronic pain, pneumonia, arthritis, ulcers, stomach pain, malaria, and women infertility [12, 13]. However, despite these ethnomedicinal uses to alleviate pain and ailments associated with inflammation and oxidative stress [12, 13], the analgesic, anti-inflammatory and antioxidant properties of extracts from different parts of *F. hildebrandtii* have not been fully investigated experimentally. Therefore, we used standard *in vivo* and *in vitro* tests to determine the anti-inflammatory, anti-nociceptive and antioxidant effects of *F. hildebrandtii* stem bark, obtained from Makueni County in Kenya.

MATERIAL AND METHODS

Sample collection and identification of plant materials

F. hildebrandtii stem bark was collected from Makueni County with help of a locally reputable herbalist who was conversant with this plant, in their natural habitat. The stem barks for the study were collected by debarking and packaged in airtight carrier bags. Aerial fertile parts of the plant were cut and used to prepare a voucher specimen that was transported to the department of botany East Africa Herbarium in Nairobi for identification and authentication. The collected stem bark was air- dried at room temperature and controlled humidity at the Department of Pharmacognosy, Mount Kenya University and later ground into dry and fine powder using an electrical motor-driven laboratory plant mill (Buchi, Switzerland AG). The powdered plant material was then kept in airtight bags awaiting water and methanol extraction.

Aqueous extraction

Water extraction was done according to methods described by Evans [14]. Briefly, 250 g of the finely powdered *F. hildebrandtii* stem bark was soaked in 500 ml distilled water and heated at 60 °C for 5 minutes. The mixture was then filtered through the Whatman filtration paper No.1 and thereafter lyophilized using a freeze drier (Thermo Fisher Scientific).

Methanol extraction

Methanol extraction was done according to the procedures described by Harborne [15]. Briefly, 250 g of the powdered stem bark *F. hildebrandtii* was soaked in 1000 ml of methanol in a 2-litre conical flask. The flask was then covered with an aluminium foil paper and gently agitated once daily for two days. Thereafter, the mixture was decanted then filtered through a Whatman filtration paper No.1 and concentrated *in vacuo* with use of a rotary evaporator (Stuart® RE300) set at 50 °C. The extract was then dried by evaporation in a closed hot-air oven (i-therm AI-7941) set at 35 °C.

Qualitative analysis of phytochemicals

The phytochemical composition of each extract was determined by previously described standard qualitative methods [14-16] with slight variation. Presence or absence was denoted by (+) and (-), respectively.

Total phenolic content of the water and methanolic extracts

Total phenolic content of the methanol and aqueous extracts of *F. hildebrandtii* stem bark was determined by Folin-Ciocalteu assay. The reaction mixture was reconstituted by mixing 500 µl of plant extracts (1000 mg/ml) or Gallic acid (Loba chemie) as standard at concentrations levels of 150µg/ml, 75 µg/ml, 37.5µg/ml, 18.75 µg/ml, 9.375 µg/ml and 4.687 µg/ml with 1500 µl 10 % Folin-Ciocalteu reagent in each test tube. Upon mixing of extract or Gallic acid with Folin-Ciocalteu (Loba chemie) reagent and incubating for five minutes, 1500 µl of 7.5% sodium carbonate (Na_2CO_3) was added, mixed by swirling and incubated in the dark for 120 minutes under room temperature. Absorbance of the reaction mixture was read at 760 nm against reagents without sample/standard as the blank using a UV-Vis spectrophotometer (Shimadzu 1601). The standard Gallic acid calibration curve was constructed using the absorbance of the various concentrations of Gallic acids used. Using the regression equation, the concentration of total phenolic in the extracts was then computed and expressed as milligram of Gallic acid equivalent per gram of dry weight (GAE, mg/g dw) using the formula:

$$\text{Concentration}\left(\text{GAE}, \frac{\text{mg}}{\text{g}}\text{dw}\right) = \frac{c \times v}{m}$$

Where **c** is the concentration from the standard Gallic acid curve, **v** is the volume of the sample and m is the mass of the extract weighed (Saeed *et al.*, 2012).

Total flavanoid content of the water and methanolic extracts

The aluminium chloride calorimetric method was used to evaluate the total flavonoid content in stem bark extracts of *F. hildebrandtii*. This briefly involved mixing 125 µl of the samples (concentration level of 1g/ml) extract or catechin (Sigma Aldrich) at concentration levels of 4000µg/ml – 12.5µg/ml with 100 µl of sodium nitrate and incubated at room temperature for six minutes. Upon completion of the incubation period, exactly 75 µl of 4% sodium hydroxide was added followed by 750 µl of aluminium chloride to make a final volume of 1000 µl. Using distilled water, this volume was made up to 2500 µl and absorbance measured at 510 nm using UV-Vis spectrophotometer double beam (Shimadzu 1601) against reagents without sample/standard as the blank. The catechin calibration curve was plotted from the absorbance versus concentration values and the regression equation obtained. Using the equation, the concentration of the total flavonoid was computed and expressed as milligrams of catechin equivalent per gram of dry weight (CE, mg/g dw) using the equation:

$$\text{Concentration}\left(\text{CE}, \frac{\text{mg}}{\text{g}}\text{dw}\right) = \frac{c \times v}{m}$$

Where **c** is the concentration from the standard catechin curve, **v** is the volume of the sample and **m** is the mass of the extract weighed [17].

Assay for anti-inflammatory activity

The anti-inflammatory activity of water and methanol extract of *F. hildebrandtii was* evaluated using Carrageenan-induced right hind paw edema mouse model as previously described by Yimer, Birru [18] with slight modifications. Briefly, 30 swiss albino male mice weighing 20 ± 2 g were randomly allocatted into 6 groups (Group A to F) containing 5 mice per group. Edema was induced through subplantar injection of freshly prepared 100 µL 1% of carrageenan in distilled water into the right hind paw of each mouse one hour after adminstration of the plant extract or negative/positive control drug. The negative control [group A] was adminstered with physiological saline *p.o*, positive control [group B] received 10 mg/Kg bw of dexamethasone *p.o.* and experimental groups C-F received the plant extracts at increasing single *p.o* doses of 2 mg/Kg bw, 10 mg/Kg bw, 50 mg/Kg bw and 250 mg/Kg bw, respectively. The linear paw circumference was measured at hourly intervals for 5 hours. The percentage edema inhibition was then calculated as previously described by Rahman, (2011).

$$\text{Percentage inhibition of edema} = \frac{\text{PECm} - \text{PETm}}{\text{PECm}} \times 100\%$$

Where; PECm is paw edema inhibition in negative control mice and PETm is paw edema inhibition in mice given plant extract or 10 mg/Kg bw of dexamethasone.

Assay for Antinociceptive activity

In this study, the acetic acid-induced writhing model as described by Koster, Anderson [19] was used to evaluate the the analgesic activity of the plant extracts. Briefly, male swiss albino mice was randomly assigned to 6 treatment groups (5 mice per group) as follows: Negative control group [A] received normal saline (0 mg/Kg bw *p.o*); positive control group [B] was given aspirin (150 mg/Kg bw *p.o*) experimental groups (C-F) received the plant extract at 2 mg/Kg bw, 10 mg/Kg bw, 50 mg/Kg bw and 250 mg/Kg bw respectively. All the mice groups was injected (*ip*) with 200 µL of 0.6 % acetic acid 30 minutes after adminstration of extract or reference drug or normal saline. The writhing frequency in each of the mice was then determined five minutes post acetic acid injection and theafter for 15 minutes. The anti-nociception effects was then expressed as percentage change in writhing in comparision with negative control as follows [20].

$$\text{Percentage inhibition of writhing} = \frac{\text{WCmA} - \text{WTm}}{\text{WCmA}} \times 100\%$$

Where; WCmA: is the average number of writhes in negative control mice and WTm is the average number of writhes in mice receiving the extracts or standard drug.

Antioxidant activity assay

The antioxidant activity of *F. hildebrandtii* extracts was evaluated using 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay as described by [21] with few modifications. *F. hildebrandtii* stem bark extracts and ascorbic acid standard (10 mg/10 ml) was prepared in concentration of 0.01µg/ml, 0.1µg/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml in methanol. DPPH (0.1 mM) was prepared in methanol and 1.6 ml added to 1.4 ml of the different concentrations of specific plant extract and that of the standard (ascorbic acid i.e. 10 mg/10ml). A mixture of 1.6 ml 0.1 mM DDPH solution in methanol (Scharlau) and 1.4 ml of methanol served as the negative control. The test samples and the standard were prepared in triplicate which was then kept in a dark closet at room temperature for approximately 15 minutes allowing reaction to take place. Absorbance was measured at wavelength of 517 nm against methanol as blank using an ultraviolet-visible spectrophotometer (Shimadzu 1601**)** in triplicates. The percentage radical scavenging activity, (%RSA) calculated using below formula [22]

$$\%\ \text{Radical Scavenging Activity} = \frac{\text{Absorbnce of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100\%$$

Data analysis

The obtained data was tabulated in Microsoft excel 365 and then analyzed using Minitab version 19.2 software and Graph pad prism version 7. Two-way ANOVA and post hoc tests was used for comparison of group means, with statistical significance set at $p \leq 0.05$.

RESULTS

Qualitative phytochemical screening of *F. hildebrandtii* extracts

Water extract as well as methanol extract of *F. hildebrandtii* exhibited presence of steroid, flavonoid, terpenoid, coumarin, flavonoids, alkaloids, tannin, saponin and glycosides, but anthocyanins and anthraquinones were absent (Table 1).

Table 1: Phytochemical content of *F. hildebrandtii* water and methanol extracts

Phytochemical	**Test**	**Inference**	
		Water extract	**Methanol extract**
Steroids	Liebermann burchard test	+	+
Phenols	Ferric chloride test	+	+
Saponins	Frothing test	+	+
Tannins	Ferric chloride	+	+
Glycosides (cardiac)	Kedde's test	+	+
Alkaloids	Dragendoff's test	+	+
	Mayer's test	+	+
	Wagner's test	+	+
Flavonoids	Conc.HCL test	+	+
Terpenoids	Salkowski's test	+	+
Coumarins	Sodium hydroxide test	+	+
Anthocyanins	Hydrochloric acid (2N) test	-	-
Anthraquinones	Borntragger's test	-	-

(+) presence; (-) absence

Table 2 indicates quantitative analysis of flavonoids and phenolics. The findings revealed higher total flavonoid levels in *F. hildebrandtii* methanolic extract compared to water extract (132.18 ± 0.26 mgCE/g vs. 42.68 ± 0.93 mgCE/g; $p<0.05$). On the other hand, *F. hildebrandtii* methanol extract had higher total phenolic content compared to the water extract (34.590 ± 2.49 mgGAE/g vs. 7.89 ± 1.62 mgGAE/g; $p<0.05$). Notably, both water and methanol extracts exhibited higher

total flavonoid content compared to total phenolic content table 2 (p = 0.001).

Table 2: Total phenolic and total flavonoid content of *F. hildebrandtii* water and methanol extracts

Phytochemical	Water extract (Mean ± SD)	Methanol extract (Mean ± SD)	P value
Total phenolic (mgGAE/g)	7.89±1.62	34.59±2.49	P<0.05
Total flavonoid (mgCE/g)	42.68±0.93	132.18±0.26	P<0.05

Data was analysed by one-way ANOVA with Fisher pair wise comparison of means. N= triplicate tests.

Antioxidant activity of *F. hildebrandtii* water and methanol extracts

The percentage radical scavenging activity (% RSA) of *F. hildebrandtii extracts* and ascorbic acid (standard antioxidant) at concentrations ranging from 0.01-1000 µg/ml was evaluated using DPPH free radical scavenging assay [21]. *F. hildebrandtii* water and methanolic extracts exhibited concentration-dependent increase in % RSA reaching a maximum of 93.64 ± 1.39% and 93.56 ± 0.9%, respectively (Table 3, p<0.05 two-way ANOVA). Comparison with the standard ascorbic acid indicated that both extracts have a higher % RSA at lower concentration ranges of 0.01-1 µg/ml (p<0.05, Turkeys test). At concentration range of 10-100 µg/ml, *F. hildebrandtii* water and methanolic extracts exhibited lower % RSA compared to ascorbic acid (Table 3, p<0.05, Turkeys test). However, there were no significant differences between the three treatments at 1000 µg/ml. The IC_{50} for the water, methanol and ascorbic acid was 0.98µg/ml, 0.987µg/ml and 5.674µg/ml (Table 3). Collectively, this data indicates that *hildebrandtii* water and methanolic extracts have potent antioxidant activity.

Table 3: Percentage radical scavenging activity (%RSA) and IC_{50} of *F. hildebrandtii* extracts, and ascorbic acid

Concentration(µg/ml)	*F.hildebrandtii Water extract* (Mean ± SD)	*F.hildebrandtii* methanolic extract (Mean ± SD)	L-ascorbic acid (Mean ± SD)
0.01	$48.81±5.59_B^a$	$49.26±3.63_C^a$	$26.51±6.77_C^b$
0.1	$52.56±7.21_B^a$	$49.57±3.65_C^a$	$30.94±2.18_C^b$
1	$55.17±5.93_B^a$	$50.62±3.23_C^b$	$33.82±2.94_C^c$
10	$54.82±6.85_B^b$	$54.54±3.23_C^b$	$88.12±6.49_B^a$
100	$66.83±5.6_B^c$	$72.88±1.87_B^b$	$95.80±0.24_A^a$
1000	$93.64±1.39_A^b$	$93.56±0.90_A^b$	$96.38±0.89_A^b$
IC_{50}	0.98	0.987	5.674

Data was analysed by two-way ANOVA with Fisher pair wise comparison of means. N= triplicate assay per concentration. Values of the same superscript within the row and values of the same subscript within the column are not significantly different.

The anti-inflammatory activities of *F. hildebrandtii* water and methanolic extracts

The anti-inflammatory effects of *F. hildebrandtii* water and methanolic extracts was determined by evaluating the hourly percentage inhibition of carrageenan-induced hind paw edema in mice over a period of 5 hours with dexamethasone as standard control drug. Overall, *F. hildebrandtii* methanol and water extracts showed a dose and time-dependent inhibition of carrageenan-induced hind paw edema that was not significantly different from dexamethasone standard (10 mg/Kg) over the 5-hour test period (Figure 1, p>0.05, two-way ANOVA). Post hoc analysis showed that edema inhibition by dexamethasone standard (10 mg/Kg) was only significantly higher compared to 2 mg/kg, 10 mg/kg and 50 mg/Kg of water extract at the 2-hour experimental time point (p<0.05, Tukey's test). Altogether, this data indicates that *F. hildebrandtii* water and methanolic extracts have good anti-inflammatory activities, with the water extract having an initial lower activity at low doses.

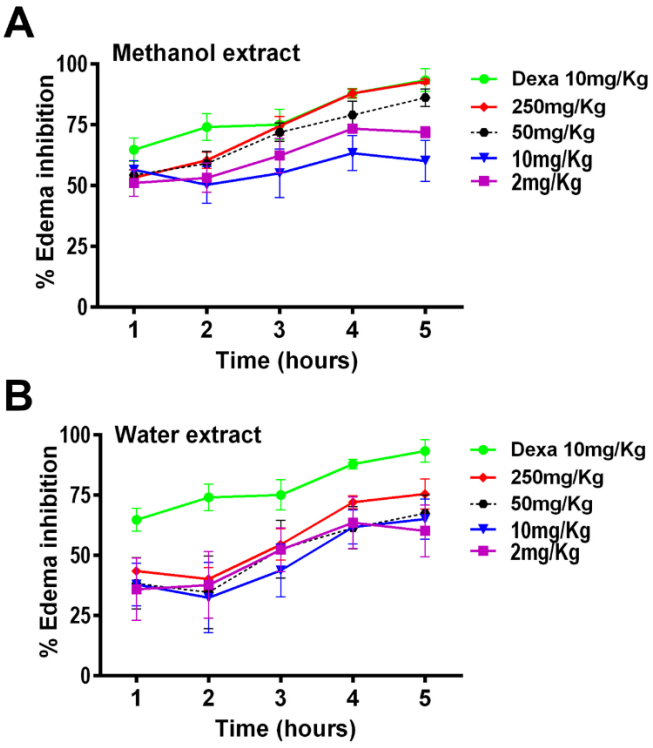

Figure 1: Percentage inhibition of edema of increasing doses of **A)** *F. hildebrandtii* methanol extract **B)** *F. hildebrandtii water* extract in comparison with dexamethasone standard (10 mg/kg). Data was analysed by two-way ANOVA and are presented as mean ± SEM.

The anti-nociceptive activities of *F. hildebrandtii* water and methanolic extracts

The anti-nociceptive effects of *F. hildebrandtii* water and methanol extracts at increasing concentration (2-250 mg/Kg, *p.o*) was evaluated by calculating the percentage inhibition of acetic acid-induced writhing in mice compared with 150 mg/kg of aspirin as standard drug. As shown in table 4, both water and methanol extracts of *F. hildebrandtii* exhibited a dose-dependent inhibition of acetic acid-induced writhing in mice, reaching a maximum of 53.1 ± 6.1% and 80.24 ± 12.59%, respectively, at 250 mg/Kg. Notably, the percentage inhibition of acetic acid-induced writhing by 50 mg/kg and 250mg/kg of *F. hildebrandtii* methanolic extract was similar to that of aspirin standard at 150 mg/kg (77.75 ± 19.64% vs. 80.24 ± 12.59% vs. 84.99 ± 3.26%, p>0.05, two-way ANOVA, Figure 2). However, 2 mg/kg and 10 mg/kg of *F. hildebrandtii* methanolic extract and *F. hildebrandtii* water extract at all the tested concentrations (2-250 mg/kg) exhibited significantly lower percentage inhibition of acetic acid-induced writhing compared to aspirin standard (150 mg/kg)

(p<0.05, two-way ANOVA, Figure 2). Our data also revealed that the methanol extract exhibited higher percentage inhibition of acetic acid-induced writhing compared to the water extract at all the tested concentrations (p<0.05, two-way ANOVA, Figure 2).

Table 4: Dose-dependent percentage writhing inhibition of *F. hildebrandtii* extracts

Dose(mg/Kg)	*F. hildebrandtii* Water extract (Mean ± SD)	*F. hildebrandtii* methanol extract (Mean ± SD)
2	27.57±16.79	56.2±17.76
10	21.07±11.77	65.57±6.98
50	36.5±8.05	77.75±19.64
250	53.10±6.1	80.24±12.59

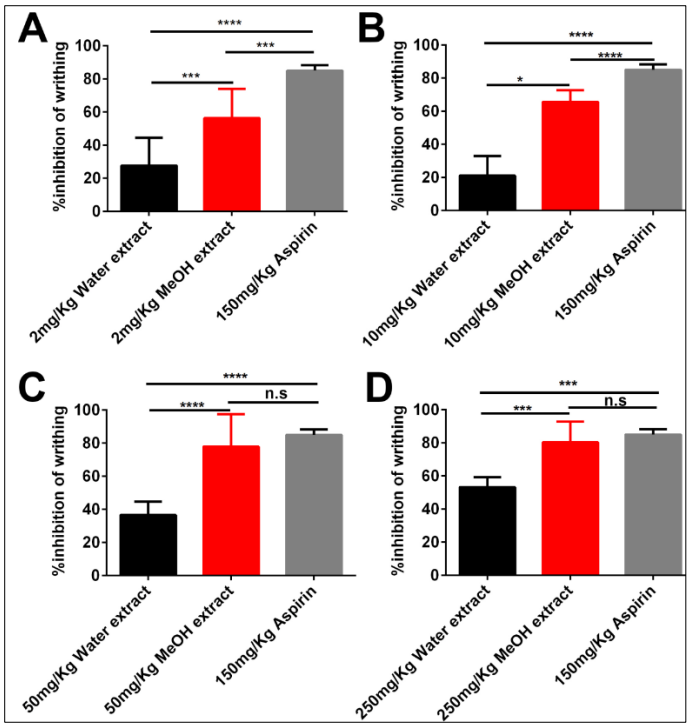

Figure 2: Percentage inhibition of writhing by increasing doses of *F. hildebrandtii* methanol and water extracts **A)** 2 mg/Kg B) 10 mg/kg, **C)** 50 mg/Kg and **D)** 250 mg/Kg in comparison with aspirin standard (150 mg/Kg). Data was analysed by two-way ANOVA and are presented as mean ± SD. MeOH: methanol.

DISCUSSION

Fagaropsis species have been widely used by various communities in Kenya, Uganda, and Ethiopia for management of ailments including malaria, respiratory diseases, joint pain, cancer and infertility [11]. These ailments are associated with pain, inflammation, and oxidative stress. Therefore, in this study we investigated the phytochemical composition, antioxidant, anti-inflammatory and anti-nociceptive properties of water and methanolic extracts of *F. hildebrandtii* stem bark using *in vitro* free radical scavenging assay, *in vivo* carrageenan-induced paw edema and acetic acid-induced pain experimental paradigms [18, 19, 21].

We show that, both water and methanolic extracts of *F. hildebrandtii* stem bark are rich in alkaloids, terpenoids, glycosides, saponins, tannins, coumarins, steroids, phenols, and flavonoids (Table 1). These findings are consistent with previous phytochemical analyses of *F. hildebrandtii* root extract [12]. Flavonoids, alkaloids, phenols, glycosides, terpenoid, tannin and steroids have also been reported to be present in the stem bark of the closely related *F. angolensis* but not saponins [23]. *F. hildebrandtii* water and methanol extracts had total phenolic content ranging from 7.89 mgGAE/g to 34.59 mgGAE/g and flavonoid content ranging from 42.68 mgCE/g to 132.18mgCE/g (Table 2), with methanol extract exhibiting a higher content. The higher total flavonoid and phenolic content of methanol extract might be due to higher solubility of *F. hildebrandtii* stem bark flavonoids and phenols in methanol than water. Previously, no studies have reported on the total phenolic content and total flavonoid content of this plant part.

Plant flavonoids and phenols have been shown to have antioxidant activities both *in vitro* and *in vivo* [24]. Consistent with this, we show that *F. hildebrandtii* water and methanol extracts exhibits a dose-dependent antioxidant activity as demonstrated by increasing % DPPH free radical scavenging activity (Table 3). Importantly, *F. hildebrandtii* water and methanol extracts had 5.7-fold lower IC_{50} compared to the ascorbic acid standard (Table 3). This indicates higher *in vitro* antioxidant potency of *F. hildebrandtii* extracts relative to ascorbic acid. Notably, however, percentage radical scavenging activity of *F. hildebrandtii* extracts was comparable to that of ascorbic acid at higher doses (Table 3), indicative of their similar maximal effect (*Emax*). Further studies should thus be done to compare the *in vivo* bioavailability and antioxidant activities of *F. hildebrandtii* and ascorbic acid or other conventional antioxidants that are currently in use. Although of the same genus, previous studies of *F. angolensis* stem bark methanol extract, reported lower antioxidant activity relative to ascorbic acid, which is opposite to our findings [23]. This could be due to difference in species, plant source, or the extraction methods, which involved use of 80% methanol followed by fractionation with various solvents. Our data demonstrate that *F. hildebrandtii* crude methanol extract at doses of 2 mg/kg to 250 mg/kg inhibited carrageenan-induced paw edema to a similar extent as 10 mg/kg of dexamethasone standard over the course of 5 hours (Figure 1A). Similarly, inhibition of carrageenan-induced paw edema by *F. hildebrandtii* water extract was not different from 10 mg/kg of dexamethasone standard, except at the 2-hour time point (Figure 1B). These data indicate potent anti-inflammatory effects of *F. hildebrandtii*, and perhaps partly explain its widespread traditional use by the local communities in the treatment of ailments associated with inflammation. Our results showing insignificant difference in percentage inhibition of edema achieved by 2 mg/Kg and 250 mg/kg of *F. hildebrandtii* extracts suggest that 2 mg/kg might be below the minimum effective dose needed to suppress carrageenan-induced inflammation, and that higher doses does not necessarily lead to increased anti-inflammatory effects.

Besides, antioxidant and anti-inflammatory activities *F. hildebrandtii* extracts also exhibited anti-nociceptive activity against intraperitoneal acetic-acid induced pain. These anti-nociceptive effects were dose-dependent; with the highest dose (250 mg/Kg) of both water and methanol extracts exhibiting the highest writhing inhibition (Table 4 and Figure 2). The inhibition of writhing by aspirin standard (150 mg/Kg) was like that of methanol extract (250 mg/Kg) and 50mg/Kg (Figure 2). The methanol extract also exhibited higher writhing inhibition compared to the water extracts at all the doses (Figure 2). This suggested higher anti-nociceptive potency of the methanol extract, which can be due to its higher flavonoid and phenolic content

(Table 2). Acetic-acid induced pain is driven by activation of peritoneal prostaglandin and mast cell inflammatory pathways [25].Therefore, the anti-nociceptive effect of *F. hildebrandtii* extracts is most likely linked to the observed anti-inflammatory activity of *F. hildebrandtii* extracts (Figure 1). However, unlike the anti-inflammatory effects, the anti-nociceptive effects were dose-dependent, suggesting potential additional and direct antinociceptive mechanisms, which warrant future mechanistic studies. Our results show that *F. hildebrandtii* could be like the conventional non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin which have both analgesics and anti-inflammatory effects. This warrant isolation and identification of active compounds as well as more detailed mechanistic studies and further controlled human studies.

CONCLUSION

In conclusion, *F. hildebrandtii* water and methanol extracts have potent antioxidant, anti-nociceptive and anti-inflammatory effects, therefore validating its use as traditional remedy by local communities in the east African region.

Abbreviations

ABS; Absorbance, ANOVA; Analysis of variance, BW; Body weight, CE; Catechin equivalent, DPPH2;2-diphenyl-2-picrylhydrazyl, GAE; Garlic acid equivalent, NSAIDs; Non-steroidal anti-inflammatory drugs, RNS; Reactive nitrogenous species, ROS; Reactive oxygen species, RSA; Radical scavenging activity.

Conflict of Interest

None declared.

Financial Support

None declared.

ORCID ID

Peter Maloba: http://orcid.org/0000-0001-8030-6824
Elias Mandela: http://orcid.org/0000-0002-8433-1477
Sammy Kimoloi: http://orcid.org/0000-0003-3364-4980
Jared Onyancha: http://orcid.org/0000-0002-2870-9471

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HOW TO CITE THIS ARTICLE
Maloba P, Mandela E, Kimoloi S, Onyancha J. Antiinflammatory, Antinociceptive and Antioxidant activities of aqueous and methanolic stem bark extracts of *Fagaropsis hildebrandtii* (Engl.) Milne-Redh. (Rutaceae). J Phytopharmacol 2023; 12(4):211-217. doi: 10.31254/phyto.2023.12401