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Phytochemical, Antioxidant, Anti-Nociceptive and Anti-Inflammatory Studies of the Water and Methanol Extracts Obtained from the Leaves of Fagaropsis Angolensis (Engl.) H.M. Gardner (Rutaceae)

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Abstract:- *Fagaropsis angolensis* is widely used in African traditional medicine system to manage oxidative stress-associated diseases and lacks scientific evidence. The study investigated the phytochemical, antioxidant, anti-nociceptive and anti-inflammatory properties of *F. angolensis* leaf extracts. Extracts were prepared by maceration and standard qualitative methods were used for phytochemical screening. Total phenolic and total flavonoid contents were done by using Folin-Ciocalteu and aluminum chloride calorimetricmethods, respectively. Antioxidant activity was evaluated using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method. Acetic acid-induced writhing and carrageenan-induced hind paw edema mouse models were used in anti-nociceptive and anti-inflammatory activities respectively. The presence of steroids, phenols, alkaloids, flavonoids, anthraquinones, glycoside and coumarins were observed. Total phenolic content for methanol extract was 55.52 ± 3.05 and the water extract was 48 ± 0.185 mgGAE/g (p<0.05). While, total flavonoid content for methanol extract was 172.53 ± 7.095 and that of water extracts was 42.23 ± 0.101 mgCE/g (p<0.05). IC_{50} values of less than 1 µg/ml were revealed in the DPPH assay. Percentage (%) writhing inhibition did not show any difference between the tested doses of plant extracts and standard aspirin at 150 mg/kg (p>0.05), indicative of potent anti-nociceptive activity. There was no significant difference in percentage paw edema inhibition between the plant extracts and the dexamethasone standard at 10 mg/kg (p>0.05), indicative of potent anti-inflammatory activity.This study adds to existing knowledge about the utilization of *F. angolensis* in traditional medicine for the management of related to oxidative stress including pain and inflammatory reactions.

Keywords:- *DPPH Assay; Medicinal Plants; Phenolic Content; Rheumatoid.*

I. INTRODUCTION

The genus *Fagaropsis* belongs to Rutaceae, a large family consisting about 154 genera and 2100 species [1]. The genus is composed of four known species with accepted names as *F. angolensis* (Engl.) H.M. Gardner, *F. hildebrandtii (*Engl.) Milne-Redh, *F. glabra* Capuron, *and F. velutina* Capuron [2]. These plant species are trees or shrubs occurring in the tropics as evergreen forests in arid and semi-arid regions. They are distributed in the Eastern, Central and Southern parts of Africa as well as Madagascar. *F. angolensis* is widely distributed in Kenya, Tanzania, Uganda, Ethiopia, Namibia, Angola and Zimbabwe [2]. *F. angolensis* and *F. hildebrandtii* are reportedly documented in Kenya. Whereby *F. angolensis* is characterized with wide distribution, including such regions as the Rift valley highlands, central Kenya highlands, Taita hills, the drier parts of Makueni, Embu and Kwale county, Kenya [3], [4], [5], [6], [7],while *F. hildebrandtii* is found mainly in Nairobi and it's the surrounding counties like Kiambu, Machakos and Makueni [7], [8], [9].

In African traditional medicine various parts of *Fagaropsis angolensis* are used in management of various diseases. The frequently used parts by the Kenya people are the roots, stem bark and the leaves. The decoction obtained from the root of *F. angolensis* is taken orally to treat cancer and malaria [3], [10], [11], [12]. The root is also chewed to serve as a cough expectorant [11], [13]. The stem bark decoction drunk for the treatment of cancer [4], chest problems, intestinal disorders [14] and malaria [12], [15]. The leaf decoction is also taken orally for treatment of malaria [16], back- ache and Joint-aches [16]. People in other African countries like Ethiopia drink the leaf or fruit decoction for treating human illnesses including and stomach-ache, the fruit decoction for rheumatism [17] and chewing of seeds to treat malaria and epilepsy[18], [19]. For livestock diseases, the leaf decoction is taken orally to treat stabbing pain and diarrhea while the fruit is also used for stomach-ache, coughs and cancer [17]. Furthermore, the stem decoction is taken orally to treat malaria, pneumonia, amoebiasis, and diarrhea [20]. In Malawi and Zimbabwe, the powder from various parts of the plant is mixed with porridge or gruel and drunk to treat male infertility (Ken, 2014). In Uganda, the root and stem bark decoctions are used for treatment of pneumonia, respiratory infections, stomach pain and snakebite in humans [21], in addition, to treating, contagious bovine/caprine pleuropneumonia in livestock [22].

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Various studies of *F. angolensis* so far have reported potential activities following the *in vitro* antimicrobial, antioxidant, cytotoxic, anticancer and antiplasmodial assays of the stem bark [21], [22], [23], [24], [25], [26], [27], [28]. Other *in vitro* studies include the antimicrobial and insecticidal potency of the essential oil mixtures from the seeds of *F. angolensis* [20]. *In vivo* studies of the extracts from the stem bark of *F. angolensis* plant have also been reported with potential antimalarial activities [25], and also acute oral toxicity [25], [27]. The extracts and compounds isolated from root bark has demonstrated low *in vitro* anticancer (Yiaile *et al.*, 2018; Mukavi, 2020), and strong anti- inflammatory potency [5]. The mosquito larvicidal activity of the leaf extracts and isolated compounds has also been evaluated [31], [32]. The phytochemistry studies of *F. angolensis* have reported isolation of compounds that are associated with the ethnomedicinal or biological activities. For instance, the norhopane and norneohopane triterpenoids from the root bark [5], the oleanane triterpenoids, alkaloids and limnoids from the stem bark [24], [28] and flavonoids, phenanthrene carboxylic acid derivative, aliphatic acid esters and essential oil mixtures [5], [29], [31], [32]. The essential oil from the seeds of *F. angolensis* contain Bicyclo (3.1.0) hexane, 4- methylene-1-(1-methylethyl) (sabinene) as the major constituent in addition to 1,4-pentadiene, p-cymene, Terpinen-4-ol and 2-undecanone [20]. However, to the best of our knowledge there are limited biological assay studies of the leaf extracts to confirm the various acclaimed uses in African traditional medicine. In the current study, we assayed the anti-nociceptive, anti- inflammatory and antioxidant activities as well as the phytochemical content of *F. angolensis* leaves to provide scientific data to substantiate the ethnomedicinal claims for the treatment of conditions-associated with pain and inflammation such as back and Joint-aches.

II. MATERIALS AND METHODS

➢ *Sample Collection and Identification*

The leaves of *F. angolensis* were collected from Michegethiu village, near Kiangombe hills in North Mbeere sub county, Embu County in Kenya with help of a local herbalist (Mr. Samuel Njue Mucungu). The leaves were packaged in airtight bags and transported to the laboratory of the Research Centre at Mount Kenya University. Taxonomic identification and authentication was done at the department of botany, East Africa herbarium at the National museums of Kenya by Mr. Geoffrey Mungai. A voucher specimen (PM/JM001/2021) was deposited at the East Africa Herbarium. The leaves were dried under the shade on the drying rack to a constant weight at the pharmacognosy laboratory. The dried leaves were thereafter ground using an electric plant mill (Buchi, Switzerland AG).

➢ *Extraction Methods*

The powdered leaves were extracted using water and methanol solvents. Briefly, 300 g of leaf powder was weighed, transferred into a conical flask and soaked in 500 ml methanol for 2 days with occasional agitation. On the third day, the mixture was decanted and filtered using Whatman filter paper No.1, after which the filtrate was concentrated using rotary evaporator (Stuart® RE300) at 50 ^{O}C. The extract was then dried in the oven (i-therm AI-7941) set at 35^{O}C. For the water extraction, 50 g of coarsely ground powdered plant material was weighed into a conical flask and boiled in 100 ml of water for thirty minutes at 50 ^{O}C. The mixture was then cooled to room temperature and then filtered using Whatman filter paper No. 1 before being lyophilized to dryness using a freeze dryer (Thermo Fisher Scientific).

➢ *Qualitative Phytochemical Screening*

The phytochemical content of the leaf extracts was determined using standard qualitative techniques as previously described with minor modifications [34], [35].

Presence or absence was denoted by (+) and (-), respectively.

➢ *Quantitative Analysis of Total Phenolic and Flavonoid Content*

Total phenolic levels were determined using Folin-Ciocalteu assay method [36], [37]. Briefly, 500 µl of 1 g/ml of each plant extract was added to 2 ml of Folin- Ciocalteu reagent (Loba chemie) and after 5 minutes, 2 ml of 6 % sodium carbonate was added. The mixture was then incubated in the dark for 60 minutes after which absorbance was read at 725 nm using UV Spectrometer (Shimadzu 1601). A standard gallic acid calibration curve of 0-150 µg/ml was constructed and the concentration of total phenolic in the extracts was then computed using regression equation and expressed as milligram of gallic acid equivalent per gram of plant extract [38].

Total flavonoid content was quantified using the aluminum chloride method [36], [37]. Briefly, one (1) gram of leaf extracts were diluted in 1 ml of distilled water, followed by addition of 200 µl of 5 % sodium nitrate. Thereafter, the mixture was allowed to react for 5 minutes in the dark. 200 µl of 10 % aluminum chloride was then added and the mixture left to react in the dark for another 5 minutes. 1 ml of 4 % sodium hydroxide was then added and topped with distilled water to 5 ml.

After 10 minutes of incubation, absorbance was read at 510 nm using UV Spectrometer (Shimadzu 1601). A standard calibration curve of catechin (Sigma Aldrich) 0- 200 µg/ml was constructed. The concentration of total flavonoid in the extracts was then calculated using a regression equation and expressed as milligram of catechin equivalent per gram of extract.

➢ *Antioxidant Activity Assay*

The 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was used to evaluate the antioxidant activity of the leaf extracts in comparison to ascorbic acid standard. Increasing concentrations (0.01µg/ml, 0.1µg/ml, 1µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml) of ascorbic acid standard and the leaf extracts were prepared. 1.6 ml of each of these preparations was then measured and mixed

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with 1.4 ml of 0.1 mM DPPH, after which the mixtures were incubated in the dark for 15 minutes. DPPH in 3 ml of methanol was included as negative control (blank). Absorbance was then measured at 517 nm using UV Spectrometer (Shimadzu1601) in triplicate (N=3). Percentage Radical Scavenging Activity (% RSA) was calculated by taking absorbance of negative control minus absorbance of extract/ascorbic acid solutions, divided by the absorbance of negative control (Eqn.1). GraphPad Prism statistical model was used to determine the IC50 of DPPH using a method described by Maloba et al in 2 mg/Kg, 50 mg/Kg and 250 mg/Kg, respectively. After

➢ *In Vivo Anti-Nociceptive and Anti-Inflammatory Studies*

- *Animals for in Vivo Studies*

Eight to twelve weeks old Swiss-albino mice of body weighing 20 ± 2 g were purchased from the Pharmacology and Toxicology laboratory, Public Health department, University of Nairobi, Kenya.. The swiss-albino mice were transported to Mount Kenya University Research Centre in rectangular laboratory cages that measured 35 cm × 25 cm × 18 cm and were kept the laboratory with 12 hr day-12 hr night cycle for 1 week for acclimatisation to the laboratory conditions before the study. Soft wood shavings were spread underneath the cages to absorb moisture. There was enough rodent pellets that was dry and clean water *ad-libitum* untill four hours to the time of experiments.

➢ *Anti-Nociceptive Activity Assay*

Acetic acid-induced writhing mouse model was used to evaluate anti-nociceptive activity as previously described [37], [39], [40]. Briefly, male Swiss albino mice weighing 20 ± 2 g were randomly grouped into 6 groups of 5 mice each. Group 1 (negative control) received normal saline *p.o*; Group 2 (positive control) received aspirin (150 mg/Kg bw *p.o*) while groups 3-6 were given the plant extracts at doses of 2 mg/Kg, 1030 minutes, all the mice were injected (*ip*) with 200 µL of 0.6 % acetic acid and writhing frequency was determined following a 5 minute-time lag for total duration of 15 minutes. The equation 2 shown was used to calculate percentage writhing inhibition (anti- nociception).

$$\%\ Writhing\ inhibition = \frac{\mathrm{WCmA} - \mathrm{WTmA}}{\mathrm{WCmA}} \times\ 100\ ....\ equation\ 1$$

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

➢ *Anti-Inflammatory Activity Assay*

The Carrageenan-induced hind paw edema mouse model of inflammation was used to evaluate the anti-inflammatory activity of *F. angolensis* water and methanol leaf extracts as previously described [41], [42], with slight modifications. Briefly, Swiss albino male mice weighing 20 ± 2 g (8-12 weeks) were randomized into 6 groups of 5 mice each. Group 1 (negative control) received normal saline *p.o*; Group 2 (positive control) received 10 mg/Kg of dexamethasone *p.o*, the remaining groups (3-6) received the plant extracts at increasing single *p.o* doses of 2 mg/Kg, 10 mg/Kg, 50 mg/Kg and 250 mg/Kg, respectively. Hind paw edema was then induced by injecting 100 µL of freshly prepared 1% of carrageenan in distilled water into the sub plantar one hour after administration of the plant extract or negative/positive control drug. The linear paw circumference was then measured using a caliper after every one hour for a total duration of 5 hours. As a measure of anti-inflammatory activity, the percentage edema inhibition was then calculated using the formula in equation 2:

$$\%\ inhibition\ of\ edema = \frac{\mathrm{PECm} - \mathrm{PETm}}{\mathrm{PECm}} \times\ 100\ ....\ equation\ 2$$

Where; PECm is paw edema inhibition in negative control mice and PETm is paw edema inhibition in mice given plant extract or positive control.

➢ *Ethical Consideration*

Ethical approval was obtained from institutional scientific and ethics review committee of Masinde Muliro University of science and Technology (MMUST/IREC/ 119/2023) and National commission for science and Technology and innovation (NACOSTI) with a license number NACOSTI/P/23/24581.

➢ *Data Analysis*

Data was tabulated in Microsoft excel 365 and then descriptive analysis done using Minitab version 19.2 software to obtain mean and standard deviation. Two- way analysis of variance (ANOVA) in Graphpad prism was then used to compare group means with statistical significance set at $P \leq 0.05$, followed by Tukey's post hoc test for multiple comparisons.

III. RESULTS

➢ *Percentage Yield and Phytochemical Screening of F. Angolensis Leaf Extracts*

The extraction of *F. angolensis* leaf using water and methanol yielded 8.13% and 16.2% w/w respectively. Phytochemical screening revealed the presence of various classes of secondary metabolites as phenols, steroids, alkaloids, cardiac glycosides, flavonoids, coumarin and anthraquinones in both water and methanol extracts. Tannins and terpenoids only appeared in the methanol extract while saponins and anthocyanins were absent in both extracts (Table 1).

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Table 1 Phytochemical content of *F. Angolensis* Water and Methanol Leaf Extracts

Phytochemical	Inference	
	Water extract	Methanol extract
Steroids	+	+
Phenols	+	+
Saponins	-	-
Tannins	-	+
Cardiac glycosides	+	+
Alkaloids	+	+
Flavonoids	+	+
Terpenoids	-	+
Coumarins	+	+
Anthocyanins	-	-
Anthraquinones	+	+

Key: (+) presence; (-) absence

➢ *Quantitative Phytochemical Screening*

The amounts of phenols and flavonoids present in the water and methanol extracts from *F. angolensis* leaf were quantified. The total phenol content of the methanolic leaf extract was found to be 55.52±3.05 mgGAE/g, which was significantly higher than 48±0.19 mgGAE/g of the water extract (p<0.05; t-test; Figure 1). Similarly, the methanolic leaf extract had significantly higher levels of total flavonoids than the water extracts (172.53±7.09 mgCE/g vs. 42.22±0.1 mgCE/g; p<0.05; t-test; Figure 1).

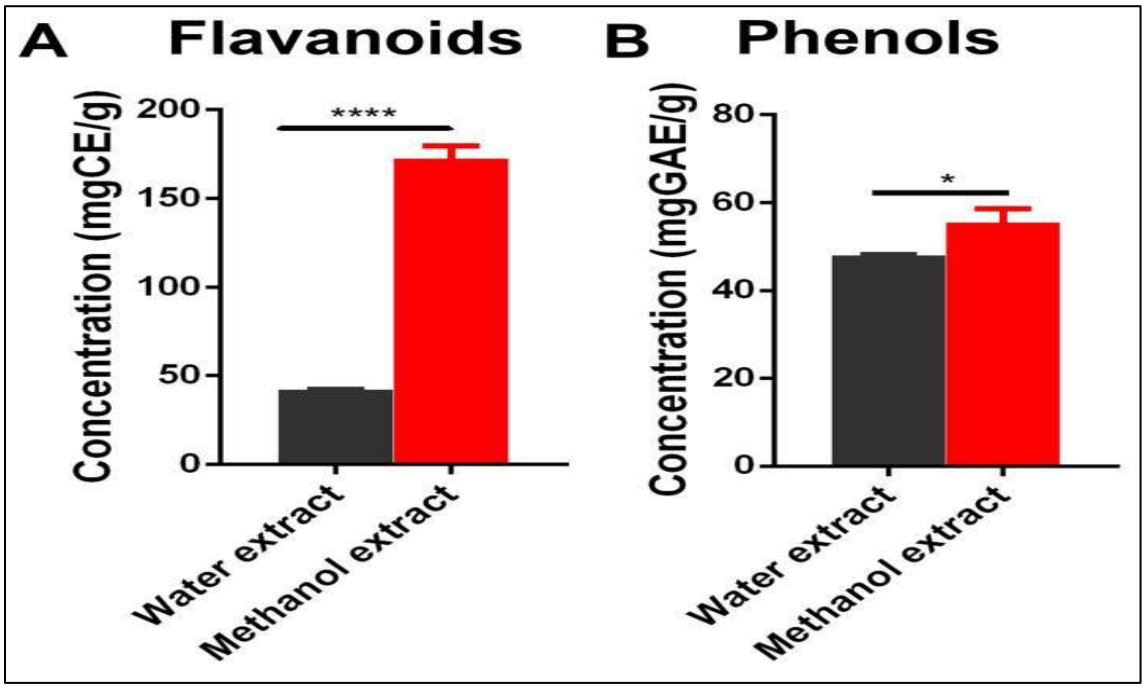

Fig 1 Total flavonoids and phenolic content of *F. angolensis* water and methanol leaf extracts. Data is presented as Mean±SD, N= triplicate tests; ****: p<0.0001, *: p<0.05; Data analysis was done by student t-test. Abbreviations: GAE-garlic acid equivalent; CE-Catechin equivalent, mg- milligram, g-gram.

➢ *In Vitro Antioxidant Activity of F. Angolensis Leaf Extracts*

To evaluate the *in vitro* antioxidant activity of *F. angolensis* leaf extracts, the % free radical scavenging activity (%RSA) of increasing extract concentrations (0.01-1000 µg/ml) was determined by DPPH method, with ascorbic acid as standard. Overall, the leaf extracts and ascorbic acid exhibited a concentration dependent increase in % RSA (p<0.0001, two-way ANOVA, Table 2). Post hoc analysis revealed that at lower concentrations range of 0.01 µg/ml to 1 µg/ml, *F. angolensis* methanolic and water extracts had significantly higher % RSA than the standard ascorbic acid (p<0.0001, Tukey's test), with no significant differences between the water and methanol extract. At the 10 µg/ml, ascorbic acid had significantly higher % RSA than both the methanolic and water extracts (p<0.0001, Tukey's test). However, at much higher concentration of 100 µg/ml and 1000 µg/ml, the % RSA of ascorbic acid standard was only significantly higher than that of water extract p<0.001 and p<0.005, respectively (Table 2), but not the methanolic extract (p>0.05, Tukey's test). The IC50 for the water extract, methanol extract and ascorbic acid were 0.01 µg/ml, 0.90 µg/ml and 5.674 µg/ml, respectively.

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Table 2 Percentage Radical Scavenging Activity (%RSA) and IC50 of *F. angolensis* leaf Extracts and Ascorbic Acid

Con. (µg/ml)	**Percentage radical scavenging activity**		
	F. angolensis Water extract (Mean %RSA±SD)	*F. angolensis Methanol* extract (Mean %RSA±SD)	L-ascorbic acid (Mean %RSA±SD)
0.00	0.00	0.00	0.00
0.01	53.25±7.58 ^{a} C	ab 47.94±3.85C	b 26.51±6.77C
0.1	54.84±7.60 ^{a} C	49.67±3.65 ^{ab} C	30.94±2.18 ^{b} C
1	56.14±8.27 ^{a} C	52.67±3.62 ^{a} C	33.82±2.94 ^{b} C
10	68.10±4.82 ^{b} B	70.47±2.04 ^{b} B	88.12±6.49 ^{a} B
100	84.06±2,36A^{b}	90.13±0.59A^{ab}	95.80±0.24A^{a}
1000	86.56±3.25A^{b}	91.85±0.56A^{ab}	96.38±0.89A^{a}
IC**50**	0.01	0.9	5.674

Data was analyzed by two-way ANOVA with Tukey's multiple comparisons test. N= triplicate assays per concentration. Values of the same superscript within the row and values of the same subscript within the column are not significantly different ($P \leq 0.05$).

➢ *In Vivo* Anti-Nociceptive Activity of *F. Angolensis* Leaf Extracts

Acetic acid-induced writhing mouse model was used to evaluate the anti-nociceptive activities of increasing *F. angolensis* leaf extracts doses (2-250 mg/Kg, *p.o*) in comparison with 150 mg/kg of aspirin as standard drug. Both the methanolic and water extracts exhibited dose- dependent inhibition of the acetic acid-induced writhing, reaching a maximum suppression of 81.95±10.76% and 83.80±6.73%, respectively, at 250 mg/Kg (Figure 2A- D). The water extract exhibited significantly lower percentage writhing inhibition at all tested doses when compared to the methanolic extract and the 150 mg/kg of the aspirin standard (p<0.05, two-way ANOVA; Tukey's test, Figure 2A-D). Notably, the writhing inhibition by methanolic extracts at doses of 50 mg/Kg and 250 mg/Kg was equivalent to that of aspirin standard dose (p>0.05, two way ANOVA; Tukey's test, Figure 2 C-D). Altogether, these results indicate potent anti-nociceptive activity of *F. angolensis* leaf extracts in mice, particularly methanol extract at doses above 50 mg/Kg.

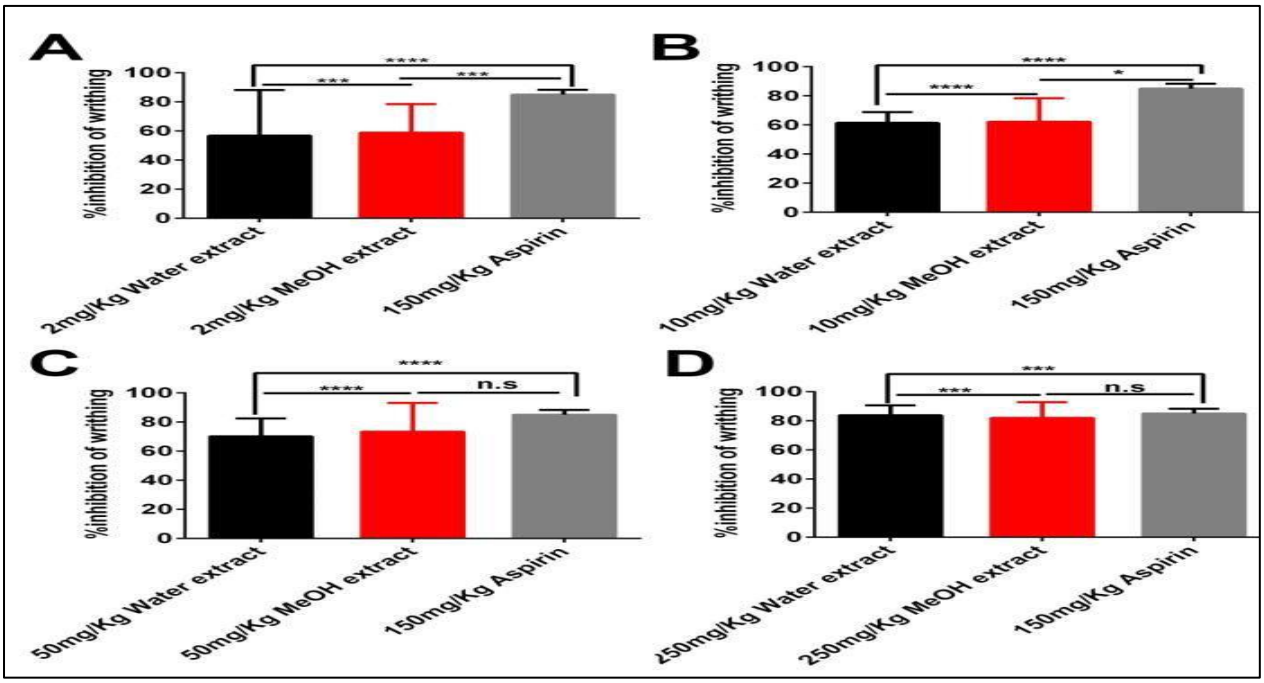

Fig 2 Percentage inhibition of acetic acid–induced writhing by increasing concentration *F. angolensis* methanol and water leaf extracts A) 2mg/Kg, B) 10 mg/kg, C) 50 mg/Kg and D) 250 mg/Kg in comparison with aspirin standard (150 mg/Kg). Data was analyzed by two-way ANOVA, with Tukey's multiple comparisons test. Data are presented as Mean ± SD; N=5 mice per group; ****: p<0.0001, ***: p<0.001 *: p<0.05, n.s: not significant. Abbreviations: MeOH-methanol; Mg-milligram; Kg-kilogram.

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- *In Vivo Anti-Inflammatory Activity of F. Angolensis Leaf Extracts*

Carrageenan-induced paw edema mouse model of acute inflammation was used to evaluate the anti-inflammatory activities of increasing *F. angolensis* leaf extracts doses (2-250 mg/Kg, *p.o*) in comparison with 10 mg/Kg, *p.o* of dexamethasone as standard drug for a total duration of 5 hours. As shown in figure 3A and 3B, the methanolic and water extracts exhibited time and dose-dependent inhibition of carrageenan-induced hind paw edema that was overall not statistically different to dexamethasone standard (10 mg/Kg) during the entire 5-hour experimental duration ($p>0.05$, two-way ANOVA, Figure 3A and 3B). However, Tukey's post hoc analysis showed that the mean percentage inhibition of edema by dexamethasone (10 mg/Kg) was superior than 2 mg/Kg of *F. angolensis* water extract at 1-hour (64.72±10.7 vs. 39 ± 29; $p<0.05$) and 2- hour (74.03 ± 12.37 vs. 47.7 ± 14.56; $p<0.05$) time points. Similarly, dexamethasone standard exhibited higher mean percentage edema inhibition compared to 2 mg/kg of methanolic extract at 1-hour (64.72 ± 10.7 vs. 33 ± 62; $p<0.05$) and 2-hour (74.03 ± 12.37 vs. 46.39 ± 16.62; $p<0.05$) time points.

Moreover, dexamethasone suppressed the edema more effectively than the 10 mg/Kg of the methanolic extract at 1-hour (64.72±10.7 vs. 36.36 ± 2.51), 2-hour (74.03 ± 12.37 vs. 44.57 ± 10.64) and 5-hour (93.33 ± 10.57 vs. 67.21 ± 16.4) time points ($p<0.05$).

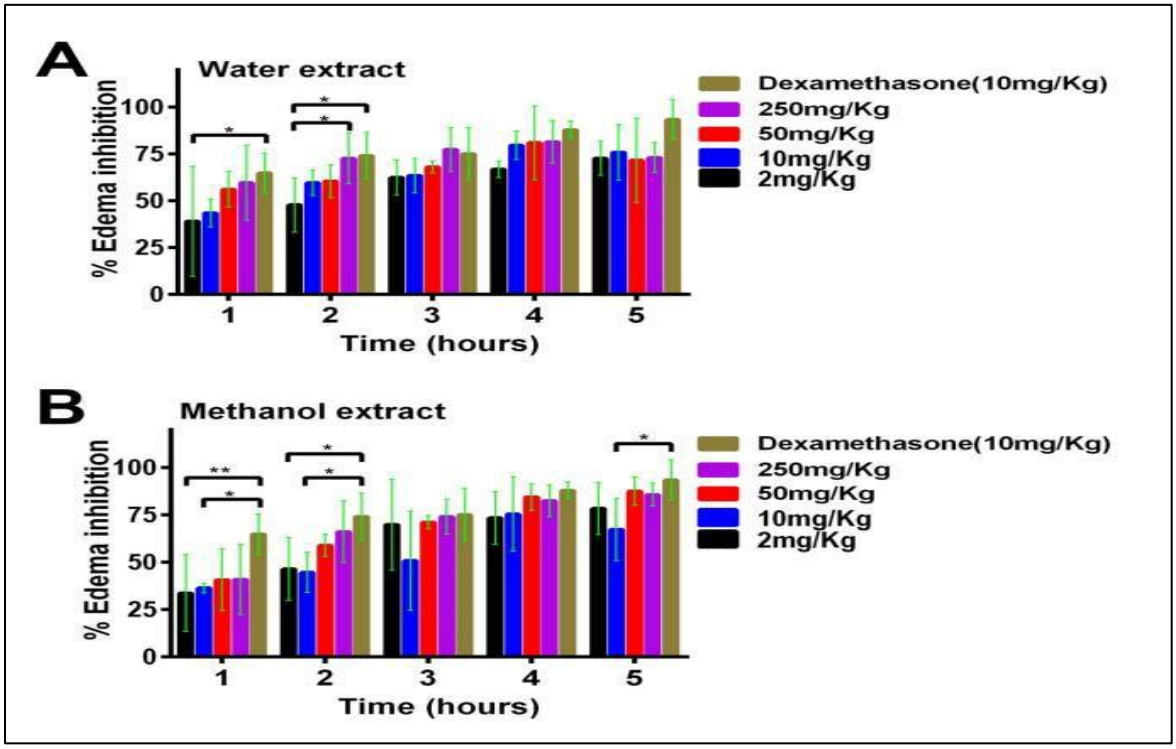

Fig 3 Percentage inhibitions of Carrageenan-induced paw edema by increasing concentration of A):*F. angolensis* leaf water *extract*, B): *F. angolensis* leaf methanol extract in comparison with dexamethasone standard (10 mg/kg). Data was analyzed by two-way ANOVA, with Tukey's multiple comparisons test. Data are presented as Mean ± SD; N=5 mice per group. Abbreviations: Mg-milligram; Kg- kilogram. **: $p<0.01$ *: $p<0.05$

IV. DISCUSSION

Several human diseases are associated with acute or chronic inflammation, pain and oxidative stress, which can be managed by conventional drugs including Cyclooxygenase (COX) inhibitors, opioids and antioxidant vitamins C and E [43]. Besides these conventional drugs, several medicinal herbs are also widely used in the management of such diseases, particularly in Africa, where more than 80 % of the population rely on herbal medicine [44]. However, unlike the conventional drugs very little is known about the pharmacological efficacy, safety and mode of action of most of these commonly used herbal medicines. Here we used *in vivo* animal models and *in vitro* free radical scavenging assay to investigate, anti-inflammatory, anti-nociceptive and anti-oxidant activities of *F. angolensis* leaf extracts for the first time.

The qualitative phytochemical screening of water and methanolic leaf extracts revealed the presence of tannins, coumarins, steroids, alkaloids, glycosides, anthraquinones, phenols and flavonoids. These results are consistent with the phytochemical profiles reported previously by Yiaile *et al*., 2018 [30] for water and methanol extracts of *F. angolensis* leaves from Embu County, Kenya. The total phenolic and flavonoid contents of the *F. angolensis* leaf extracts are also reported for the first time in this study, with our results showing that the methanolic leaf extract has a significantly higher total phenolic and flavonoid content compared to water extract (Figure 1). We show that *F. angolensis* water and methanol extracts has dose dependent *in vitro* antioxidant activity as demonstrated by increasing DPPH % RSA (Table 2). Notably, *F. angolensis* extracts exhibited lower IC_{50} (0.01 µg/ml to 0.90 µg/ml)

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compared to ascorbic acid (5.674 µg/ml). *F. angolensis* extracts also exhibited higher % RSA at lower doses, but surprisingly the water extract % RSA was lower than that of ascorbic acid at higher doses.

This indicated that *F. angolensis* extracts had a higher anti-oxidant potency than ascorbic acid standard, which in turn had a maximal efficacy (*Emax*) that was equivalent to methanol extract, but higher than that of water extract. The potent antioxidant activities of *F. angolensis* extracts may be attributed to the presence of phenols and flavonoids (Figure 1), which has been shown to have antioxidant activities. The higher concentration of phenols and flavonoid in the methanol extract compared to water extract (Figure 1) possibly explains why the latter had a lower % RSA compared to ascorbic acid at higher concentrations. To the best of our knowledge, the antioxidant activity for *F. angolensis* leaf extracts are reported for the first time. However, the stem bark extracts of the plant has revealed scavenging potency against DPPH as described in separate studies by Alemu and Misganaw [25] and Kuglerova[22]. The antioxidant activities of the water and methanol extracts from *F. angolensis* can be ascribed mainly to the presence of phenolic compounds in addition to alkaloids and terpenoids[45], [46], [47], [48].

The acetic acid-induced pain model was used to assess the peripheral analgesic activity of *F. nagolensis* methanol and water leaf extracts. The method is rapid, reliable and sensitive in testing analgesic activity of plant extracts. Acetic acid is responsible for the reactions that lead to the release of various endogenous noxious and pro-inflammatory biochemical mediators that are responsible for causing pain, such as histamine, serotonin, and bradykinin, which are associated with causation of inflammatory reactions[49]. The current study revealed potent anti-nociceptive activity of *F. angolensis* leaf methanolic extract at 50-250 mg/kg that was similar to 150 mg/kg aspirin (Figure 2). The methanol extract, at 10 and 50 mg/kg body weight of mice depicted anti-nociceptive activity that was similar in strength to that of 150 mg/kg of aspirin. This *F. angolensis* mediated anti-nociception can be attributed to the compounds, probably the flavonoids and steroids which could inhibit pain biochemical mediators from cells lining of the peritoneal cavity of the experimental mice [50].

Except for the *in vitro* anti-inflammatory activity report of the compounds isolated from the roots of *F. angolensis*, there is no available data on the *in vivo* anti- inflammatory activities of extracts from any parts of this plant [5]. Therefore, the inhibition of Carrageenan- induced paw edema was observed with the test extracts (*F. angolensis* methanolic and water extracts) in this study for the first time. The Carrageenan-induced paw edema model is well known model for evaluating the extracts with acute inflammatory activities [51]. Carrageenan is an algal polysaccharide that induces paw edema in a biphasic manner, with the first (early) phase occurring between 0-1 hour and the second (late) phase occurs at 1-4 hours post sub-plantar injection. The early phase is majorly driven by increased production of bradykinin, hydroxytryptamine and histamine, while the late phase is due to COX-2 activation, which leads to increased release of prostanoids and production of free radicals. Of note is that plant steroids and sterols are known to inhibit the synthesis and secretion of inflammatory mediators, mainly via inhibition of phospholipase A2 which is upstream of COX, as well as by inhibiting synthesis of polypeptide inflammatory mediators. Plant flavonoids are also known to inhibit inflammation via several mechanisms including inhibition of phospholipase A2, COX enzymes, and lipoxygenase, which reduces the synthesis of inflammatory prostanoids and leukotrienes. Moreover, flavonoids and phenols quench the free radicals produced during inflammation. Therefore, the steroids, flavonoids and phenols present in *F. angolensis* leaf extract (Table 1) may have contributed to the observed *in vivo* anti-inflammatory effects either individually or synergistically.

V. CONCLUSION

F. angolensis water and methanol extracts have secondary metabolites associated with antioxidant, anti-nociceptive and anti-inflammatory effects. It can therefore be concluded that the current findings are useful in the validation of the utilization of the plant extracts as in the treatment of diseases associated with oxidative stress and in this case inflammatory pain indicated by herbal practitioners. The results of the current study provide scientific data that validate the claims by traditional medicine practitioners in the treatment of pain of the body joints and the back [16]. Accordingly, more detailed future studies are needed to elucidate the identity of the antioxidant molecule (s) in different parts of *F. angolensis* and their mechanisms of action both *in vivo* and *in vitro.*

- *Data Availability*

All data are found within the manuscript or additionally upon request from the corresponding author.

- *Conflict of Interest*

The authors declare that there is no conflict of interest regarding this manuscript.

- *Authors' Contribution*

Dr. Jared Onyancha, Dr. Denis Menge and Dr. Sammy Kimoloi conceived the research idea. Peter Maloba performed the experiment and drafted the manuscript. Sydney Wanjiru helped in analysis of data. All authors reviewed and approved the final manuscript for publication.

- *Abbreviations*

Abs; Absorbance, ANOVA; Analysis of variance, CE; Catechin equivalent, CONC; Concentration, COX; Cyclooxygenase, DPPH; 2,2-diphenyl-2-picrylhydrazyl, GAE; Gallic acid equivalent, RSA; Radical scavenging activity.

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