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Comparative Analysis of Anti-inflammatory, Analgesic, and Antioxidant Properties of *Fagaropsis hildebrandtii* and *Fagaropsis angolensis* Extracts

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ABSTRACT

Fagaropsis hildebrandtii and Fagaropsis angolensis are widely used across East, Central and Southern parts of Africa as traditional remedies for management of various disorders that are associated with inflammation, pain and oxidative stress. However, it remains unclear whether F. hildebrandtii and F. angolensis plant parts have equivalent efficacy. Therefore, the objective of this study was to compare the anti-inflammatory, analgesic and antioxidant properties of the two medicinal plants. This was an experimental controlled research design. The study area was Embu and Makueni Counties. Methanol and water extracts of powdered F. hildebrandtii stem bark and F. angolensis leaves were prepared by maceration. Different extract concentrations were then tested for anti-inflammatory, analgesic and antioxidant activity using carrageenan-induced paw edema model, acetic acid-induced writhing model and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical assay, respectively. Comparative analysis revealed that F. hildebrandtii stem bark extract had higher anti-inflammatory activity compared to F. angolensis leaves (P<0.05). However, F. angolensis leaves exhibited significantly greater analgesic activity than F. hildebrandtii stem bark extracts (P<0.05). Comparison of antioxidant activities of the extracts did not show any significant differences (P>0.05). In conclusion, the findings indicate variation in analgesic and anti-inflammatory properties, but not the antioxidant properties of F. hildebrandtii and F. angolensis. Study recommends plant preservation, active pharmaceutical ingredient identification, testing in non-human primates and in clinical trials.

Key words: Anti-Inflammatory, Analgesic and Antioxidant, *F. angolensis, F. hildebrandtii*

I. INTRODUCTION

Inflammation, pain and oxidative stress occur in many communicable and non-communicable diseases and often require pharmacotherapeutic management. Current pharmacotherapeutic management of inflammation and pain involves stepped use of conventional drugs including, paracetamol (acetaminophen), non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroid and opioids (Farmer, 2020; Monteiro & Steagall, 2019; Slater et al., 2010). Although these conventional analgesics and anti-inflammatory drugs are effective, they are associated with a myriad of side effects including NSAID-induced gastrointestinal bleeding (Danelich et al., 2015; Slater et al., 2010) and opioid dependence (Veilleux et al., 2010; Voon et al., 2017).

Oxidative stress is currently treated with antioxidants including vitamins (Forni et al., 2019). Additionally, several medicinal herbs are also widely used in the traditional management of pain, inflammation and oxidative stress in different parts of the world (Ekor, 2014). In the Eastern and Southern parts of Africa, *Fagaropsis hildebrandtii* (Engl.) Milne-Redh and *Fagaropsis angolensis* (Engl.) H.M. Gardner are commonly used to treat various illnesses associated with pain, inflammation and oxidative stress (Mutinda et al., 2022). *F. angolensis* is widely distributed in Kenya, Tanzania, Uganda, Ethiopia, Namibia, Angola and Zimbabwe (Mutinda et al., 2022). In Kenya, *F. angolensis* is found in the lowlands of Central Kenya and has been reported to occur in Nandi county (Jeruto et al., 2010). *F. hildebrandtii*

is widely distributed in the Eastern parts of Africa especially, Somalia, Ethiopia and Kenya, where it is mainly found in Makueni and Machakos Counties.

Ethnomedicinal surveys shows that boiled stem bark of *F. angolensis* is used in Nandi as a traditional remedy for joint pain, respiratory system infection and back ache (Kimutai et al., 2016). *F. angolensis* leaves and stem bark are also used to treat malaria and back pain among the Embu, Meru and Mbeere communities in Eastern Kenya (Kareru et al., 2006; Muthaura et al., 2007). In Ethiopia, the leaf or fruit decoction is a traditional remedy for stomach-ache, rheumatism, malaria and epilepsy (Asnake et al., 2016; Birhan, 2022). Moreover, the stem decoction is used to treat malaria, amoebiasis and pneumonia (Kuglerova et al., 2011). *F. hildebrandtii* plant parts are also widely used in the management of various diseases that are associated with inflammation and pain in Kenya. In Makueni County, for instance, the Kambas uses *F. hildebrandtii* roots to manage, pneumonia, chronic pain, arthritis, stomach pain, ulcers, women infertility and malaria (Wambugu et al., 2011). Based on these ethnomedicinal findings, several pharmacological and phytochemical studies of *F. angolensis and F. hildebrandtii* plant parts from different regions have been reported in the recent times.

In vitro, studies of *F. angolensis* stem bark have demonstrated potent antimicrobial, antioxidant, cytotoxic, anticancer and anti-plasmodial properties (Alemu & Misganaw, 2020; Kirira et al., 2006; Onyancha et al., 2018). *F. angolensis* stem bark extracts have also been reported to have antimalarial activities. Moreover, root bark has potent anti-inflammatory properties (Mutinda et al., 2022). *In vitro* studies of *F. hildebrandtii* roots have also revealed antimicrobial activity (Mutinda et al., 2022). However, comparative pharmacological studies of *F. angolensis* and *F. hildebrandtii* plant parts are yet to be reported. In the current study, we report on the comparative analyses of anti-inflammatory, analgesic and antioxidant activities of *F. angolensis* leaves and *F. hildebrandtii* stem bark methanol and water extracts.

1.1 Statement of the Problem

Fagaropsis spp. plant parts are widely used interchangeably in the traditional treatment of various diseases that are associated with inflammation, pain and oxidative stress. This is based on the unsubstantiated assumption that *Fagaropsis* spp. and/or their different plant parts have the same pharmacological efficacy. In Kenya, there is identical use of *F. angolensis* and *F. hildebrandtii* in local traditional medicine to treat back pain, joint pain, stomachache, rheumatism, stabbing pain and respiratory diseases (Muthaura et al., 2007; Jeruto et al., 2010; Wambugu et al., 2011; Mutinda et al., 2022). In some cases, these medicinal plants are mixed with others or used alone to manage a single, and at some point, several conditions. For instance, among the Kamba community in Kenya, *F. hildebrandtii* is mixed with *Strychinos henningsii* leaves and *Carrissa spinarum* root bark in management of pain related conditions (Wambugu et al., 2011). To understand the variation in bioactivity between *F. angolensis* and *F. hildebrandtii*, there was need for a comparative study of two plants.

1.2 Research Objective

The objective of this study was to compare the anti-inflammatory, analgesic and antioxidant properties of *Fagaropsis angolensis and Fagaropsis hildebrandtii.*

II. LITERATURE REVIEW

Pain is an unpleasant sensory and emotional experience that is associated with injury or tissue damage. During harmful stimuli, pain sensation warns us to withdraw from the injury. This is accomplished through nociception, the neural processing of harmful stimuli. It is difficult to quantify pain because pain is both an affective and a sensory component (Nalamachu, 2013). Pain interferes with various aspects of life, such as social life, mental and physical health (Duenas et al., 2016).

Globally, 20% of the world population has shown both physical and emotional symptoms of pain. In the US, one in five adults had chronic pain in 2016 and 8% had high impact chronic pain (Kuehn, 2018; Salaffi et al., 2018). There was 16.6% prevalence of pain in Spain with one in four affected in each home (Dueñas et al., 2016; Zelaya et al., 2020). In south Africa, 21.5% of patients attending chronic care clinic had chronic pain. Among those patients, 18% of patients were adults (Kamerman et al., 2020). At Kakamega teaching and referral hospital, averaged between the Numerical Rating Scale (NRS) and Faces Pain Scale-Revised (FPS-R), 80.5% of patients endorsed a non-zero level of pain and 30% of patients reported moderate to severe pain. The prevalence of pain among HIV/AIDs patients in South Africa, Uganda and Kenya was at 59% to 98% (Huang et al., 2013).

Currently, acute or moderate pain is treated with various drugs including NSAIDs, paracetamol, antidepressants, gabapentin and opioid analgesics. For severe pain, long term opioids are used. Neuropathic pain management involves the use of antidepressants such as amitriptyline, calcium channel inhibitors like gabapentin/pregabalin and topical

lidocaine (Queremel and Davis, 2023). Although these drugs are relatively effective, they are sub-optimal as they are associated with several side effects including hepatotoxicity (NSAIDs) and urinary retention (amitriptyline). Some like opioids are not easily accessible and can cause respiratory depression and dependency among other side effects. Moreover, some forms of cancer-related pain and migraine-related headache do not response to the currently available analgesics (Nalamachus, 2013).

Inflammation is an adaptive response to either infection or tissue damage. The role of inflammation is restorative and healing. In certain cases, like autoimmune disease, the immune system acts as if normal tissue is infected or somehow unusual, causing damage. There are two types of inflammation, acute and chronic inflammation. Acute inflammation is a self-limiting process unlike chronic inflammation which could be destructive. The five cardinal signs of inflammation are; pain, heat, redness, loss of function and swelling (Mendes et al., 2018).

Inflammatory conditions, particularly due to chronic infectious diseases and non-communicable diseases (NCDs) like obesity, cancer, arthritis, inflammatory bowel diseases and skin diseases present with multi- organ failure. This chronic inflammation leads to tissue damage, systemic pathological changes, hypotension, increased morbidity and low quality of life and ultimately contributes to death. 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 (Furman et al., 2019). During Covid-19, around 4,000 Kenyan died among 180,000 reported cases of severe acute respiratory syndrome. Those death cases were related to respiratory inflammation, with difficulty in breathing (Brand et al., 2021).

Currently, the main pharmacological therapy for inflammation includes non-steroidal anti-inflammatory drugs that include aspirin, oxicams, diclofenac, ibuprofen and corticosteroids such as dexamethasone, hydrocortisone and prednisolone. However, these drugs have several shortcomings, including side effects and high cost. Long term use of high dose aspirin and ketorolac for example, is associated with gastrointestinal erosion and interaction with other drugs including beta blockers. Dexamethasone causes bone weakening particularly in the elderly who are already predisposed to bone resorption (Punchard et al., 2014).

Free radicals are highly reactive atoms or molecules with one or more unpaired electrons in the outer shell and are formed when oxygen interacts with molecules like nitrogen, chloride, bromide and hydrogen. Oxidative stress can be classified into four types: high, intermediate, low and basal. High oxidative stress is cytotoxic, intermediate/low oxidative stress is a stress signal, while basal oxidative stress is considered physiological (Magnani et al., 2020).

During aging and disease states, the generation of oxidants from endogenous sources is enhanced, leading to oxidative stress. Increased production of oxidants can also be induced by exogenous factors including ultraviolet (UV) light, gamma rays' radiation, electromagnetic field, alpha particle emitted by radioactive elements decay, tobacco smoke, ozone, and iron overload (Kruk et al., 2019). Importantly, oxidative stress drives tissue damage, plays a role in pathogenesis of various diseases related to both inflammation and pain (Liguori et al., 2018).

Currently available antioxidants including nutraceutical ascorbic acid, tocopherol, resveratrol and phenolic compounds are prescribed at various stages of disease progression, some as preventive while others as treatment. In the former case, antioxidants are widely used as adjuvants in the management of various health conditions including inflammation and pain. However, these antioxidants might not reach pharmacologically active concentration *in vivo*. Furthermore, these nutraceuticals neither eliminate the primary cause of disease nor limit its progression (Forni et al., 2019). Therefore, continuous search and development of novel antioxidants, analgesic and anti-inflammatory agents remain an important goal if we are to improve inflammatory pain pharmacotherapy. Apart from these contemporary drugs, there exist medicinal plants that are also used in management of pain, inflammation and oxidative stress.

The use of medicinal plants in management of various diseases in human being dates centuries ago when mankind discovered therapeutic potential of plants. Among the factors contributing to high intake of these medicinal plants is acceptability, tolerance and availability. Despite over 80% of human population, especially in Africa utilizing medicinal plants, only a few have been investigated to ascertain their ascribed therapeutic effects (World Health organization [WHO], 2019). Indeed, there is a need for experimentally controlled data on the ethno-pharmacological properties of various widely used medicinal herbs.

The genus *Fagaropsis* consist of at least four known species namely, *F. hildebrandtii* (Engl.) Milne-Redh, *F. angolensis* (Engl.) H.M. Gardner, *F. glabra Capuron*, and *F. velutina Capuron* (Mutinda *et al.,* 2022). Among these species, only *F. angolensis* and *F. hildebrandtii* species have been sited in Kenya. Ethnomedicinal evidence indicates that both *F. angolensis* and *F. hildebrandtii* are used to treat illnesses associated with pain, inflammation and oxidative stress. For instance, among communities living in Nandi County Kenya, boiled stem bark of *F. angolensis* is used in treatment of joint pain, respiratory system infection and backache (Kimutai *et al.,* 2016). In Makueni and Machakos Counties, the Kamba community in Kenya uses the roots of *F. hildebrandtii to* treat arthritis, pneumonia, stomach pain, and backache (Wambugu et al., 2011; Muia et al., 2020).

This interchangeable use of *Fagaropsis* species in traditional medicine practice has evoked interest in understanding pharmacological activity differences between *F. angolensis* and *F. hildebrandtii*. Ethnopharmacological studies of the species *F. angolensis* stem bark has confirmed ant-plasmodial activity, anti-proliferative activity, antimicrobial activity and antioxidant activity (Kimutai et al., 2016; Onyancha et al., 2018; Alemu and Misganaw 2020). Notably, *F. hildebrandtii* roots have been investigated and found to have antimicrobial activity (Muia et al., 2020). In one *in vitro* study, the root bark of *F. angolensis* exhibited *in vitro* anti-inflammatory activity, which was ascribed to inhibition of pro-inflammatory cytokines in lipopolysaccharide stimulated peripheral blood mononuclear cells by plant norhopanes (Mukavi et al., 2020). The stem bark of *F. angolensis*, 80% ethanol extract and fractionated 80% methanol extract from Uganda and Ethiopia respectively, has been shown to exhibit antioxidant activities (Kuglerova et al., 2011; Alemu and Misganaw, 2020).

The purpose of this study was to compare the bioactivity of *F. angolensis* and *F. hildebrandtii* in management of pain, inflammation and oxidative stress. The study findings confirmed the presence of different *in vivo* anti-inflammatory and analgesic activities of *F. angolensis* leaves and *F. hildebrandtii* stem bark as well as *in vivo* antioxidant activity

III. METHODOLOGY

3.1 Study Site

Leaves of *F. angolensis* (Figure 2 A-B) were collected from Michegethiu village near Kiang'ombe hills in North Mbeere sub-County, Embu County in Kenya (031.545S; 03727.112E, Figure 1). Embu County is found in the former Eastern province. It borders Kirinyaga County to the West, Kitui County to the East, Tharaka Nithi County to the North and Machakos County to the South. The County lies at 1750m above sea level. The area has an annual rainfall of 1206 mm and humidity of 70%. In Meru community, *F. angolensis* is called, "Mukuriampungu" while in Embu community it's called "Mukuria Hungu". Stem bark of *F. hildebrandtii* (Figure 2 C-D**)** was collected in Makueni County, Kilome sub-County, Kasikeu ward, Uvaleni location in Kythani village in Kenya (15250.2S; 372225.3E; Figure 1). The County is also found in the former Eastern province and borders Kajiado County to the West, Taita Taveta County to the South, Kitui County to the East and Machakos County to the North. The County lies at 1787 m above sea level. The area has an annual rainfall of 800 mm – 900 mm and humidity of 68%. Among the Kamba, *F. hildebrandtii* is locally called "Muvindavindi". Voucher specimen was prepared by packaging plant parts in airtight carrier bags and transported to the department of botany East Africa Herbarium in Nairobi for identification and authentication.

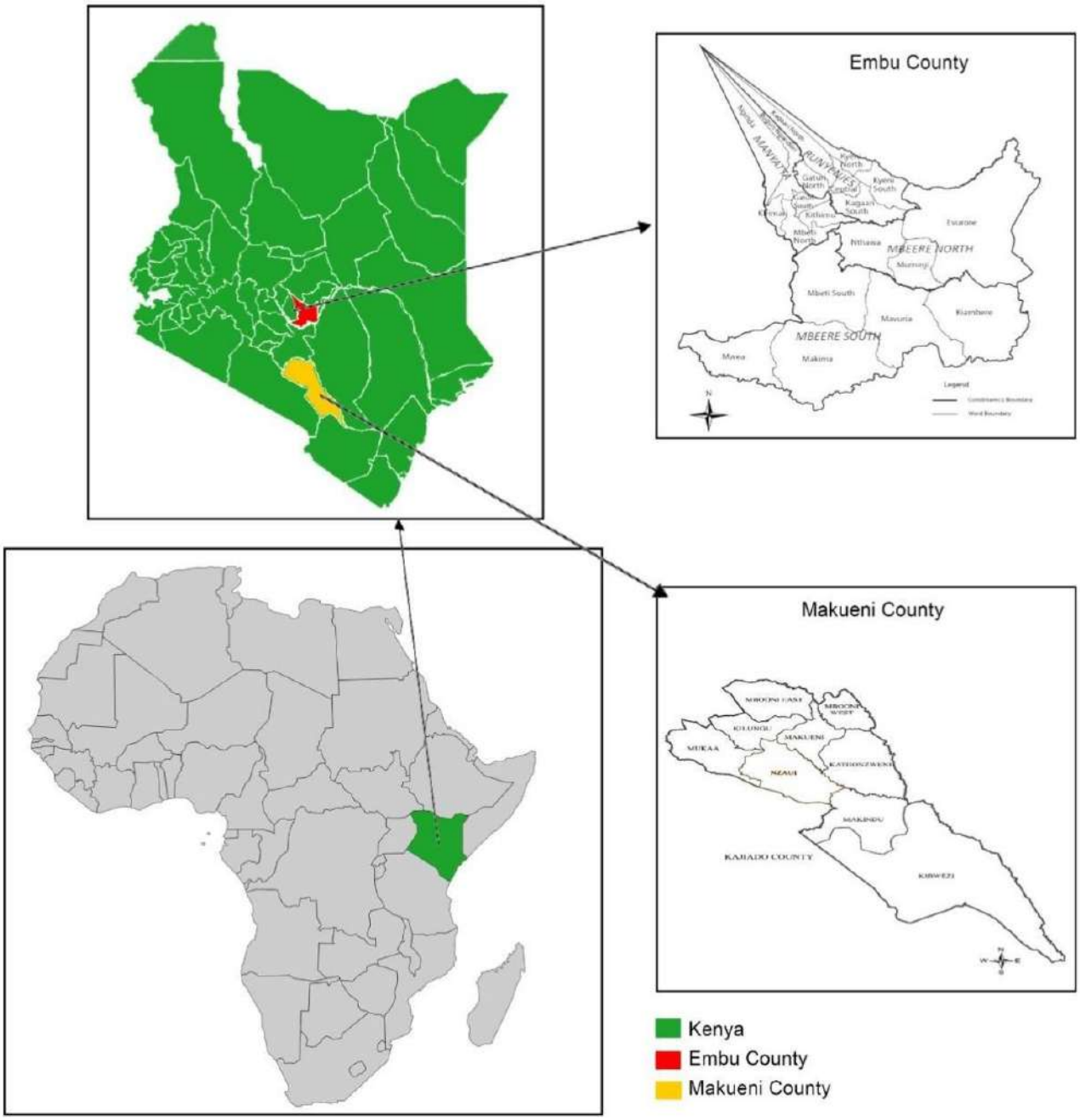

Figure 1
Map Showing the Plant Sample Collection Sites

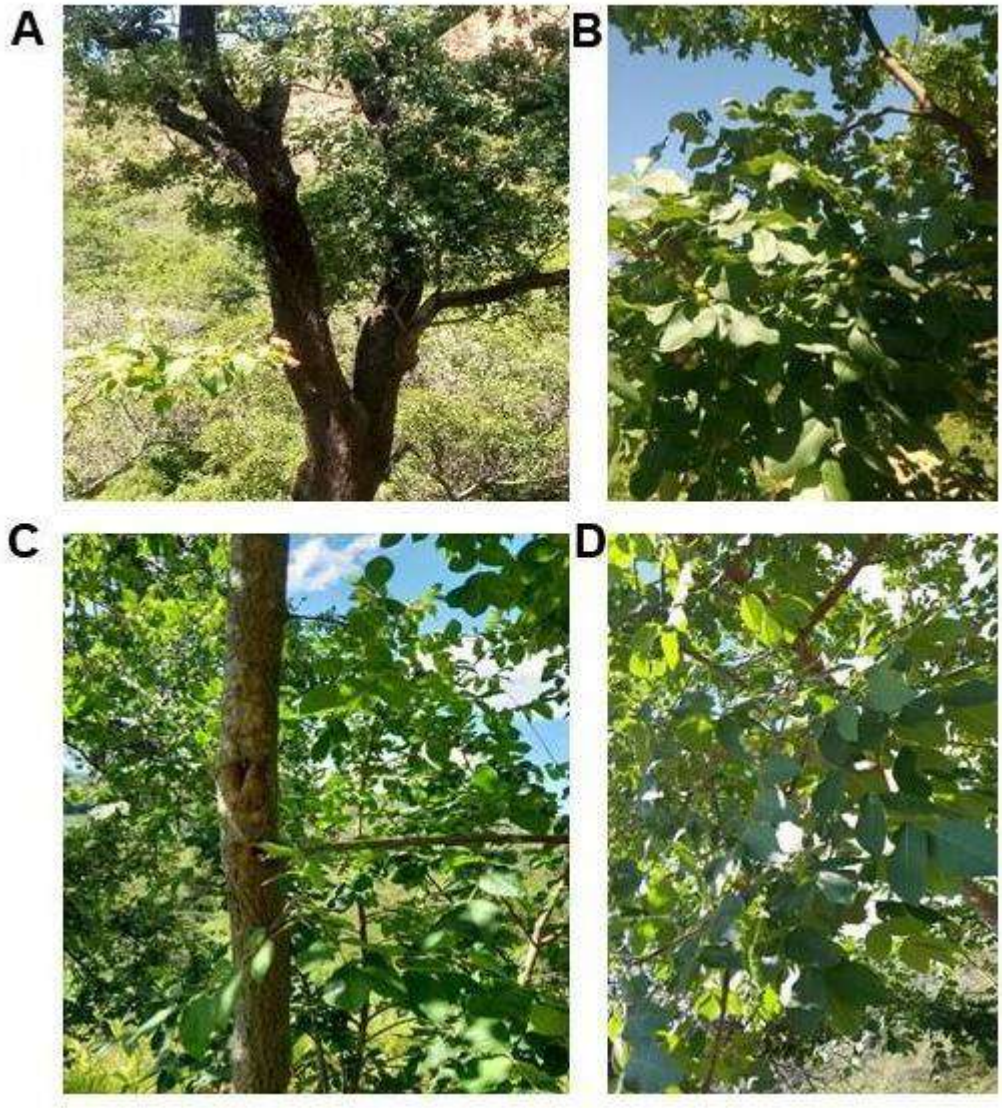

Figure 2
Photographs of the Studied Plants Species. ***A-B****) Fagaropsis angolensis* **C-D)** *Fagaropsis hildebrandtii*

3.2 Study Design

This was an experimental controlled design; Swiss albino mice were used for *in vivo* evaluation of anti-inflammatory and analgesic activity.

3.2.1 Laboratory Procedures

Water and methanol Extraction: The collected plant parts were air-dried at room temperature of 20 °C and humidity of 45 % 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. Water extraction was done according to methods described by (Evans, 2016). Briefly, 250 g of the finely powdered *F. angolensis* leaves, and *F. hildebrandtii* stem bark was soaked in 500 ml distilled water for 2 days and on third day, it was 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 dryer (Thermo Fisher Scientific). For methanol extraction, 250 g of the powdered leaves and stem bark of *F. angolensis and F. hildebrandtii* respectively were soaked in 1000ml 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.

Assay for anti-inflammatory activity: The anti-inflammatory properties of *F. angolensis and F. hildebrandtii* water and methanol extracts were assayed using carrageenan-induced right hind paw edema mouse model (Yimer et al., 2020). Briefly, 30 Swiss albino male mice weighing 20 ± 2 g were randomly allocated into 6 groups (Group A to F) containing 5 mice per group. Edema was induced through sub plantar injection of freshly prepared 100 µl 1 % of carrageenan in distilled water into the right hind paw of each mouse one hour after administration of the plant extract/dexamethasone standard/normal saline. The negative control [group A] was administered with physiological saline *p.o*, positive control [group B] received 10 mg/Kg body weight of dexamethasone *p.o.* and experimental groups received the plant extracts at increasing single *p.o* doses of 2 mg/Kg body weight, 10 mg/Kg body weight, 50 mg/Kg body weight and 250 mg/Kg body weight, 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 et al. (2011).

Assay for analgesic activity: In this study, acetic acid-induced writhing model as described by Rashid et al. (2015) was used to evaluate the analgesic activity of the plant extracts. Briefly, according to OECD 423 guideline, male Swiss albino mice was randomly assigned to 6 treatment groups (5 mice per group) as follows: Negative control group [A] received normal saline (*p.o*); positive control group [B] was given aspirin (150 mg/Kg body weight *p.o*) experimental groups (C-F) received the plant extract at 2 mg/Kg body weight, 10 mg/Kg body weight, 50 mg/Kg body weight and 250 mg/Kg body weight respectively. Dose levels were selected based on acute toxicity study previously done by Muia et al. (2020) and Alemu and Misganaw (2020). All the mice groups weighing 20 g were injected (*ip*) with 60 µl/kg of 200 µl acetic acid, 30 minutes after administration of plant extract/aspirin standard/normal saline. The writhing frequency in each of the mice was then determined 15 minutes post acetic acid injection. A reduction in the number of writhing of mice given plant extract when compared to the control group was considered as evidence of analgesic potential. The analgesic activity of plant extracts and standard aspirin was expressed as percentage change in writhing in study groups compared to the negative control (Cheng et al., 2016).

Assay for antioxidant activity: The antioxidant activity of *F. angolensis* and *F. hildebrandtii* extracts was evaluated using 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay as described by Moriasi et al. (2020) with few modifications. *F. angolensis* and *F. hildebrandtii* leaves and stem bark extracts, respectively and ascorbic acid as standard (1mg/ml) were prepared in concentration of 0.01 µg/ml, 0.1 µg/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml in methanol. Thereafter, 1.4 ml extract/ascorbic acid standard was transferred into cuvette containing 1.6 ml of DPPH (0.1 mM) prepared in methanol. A mixture of 1.6 ml 0.1 mM DPPH 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) was calculated and IC_{50} computed from the graph of concentration against % RSA (Rajurkar & Hande, 2011).

3.3 Statistical Analysis

Raw data was tabulated in Microsoft excel version 365 and then descriptive analysis was done using Minitab version 19.2 to obtain mean and standard deviation. One-way ANOVA was then used to compare group means with statistical significance set at $P \leq 0.05$.

IV. FINDINGS & DISCUSSIONS

4.1 Comparative Analysis of Anti-Inflammatory Properties

The percentage inflammatory inhibition for the two plant species was evaluated against standard dexamethasone 10 mg/kg for a total of 5 hours post carrageenan administration. The results shows that a 250mg/kg dose of *F. hildebrandtii* methanol extract had significantly higher percentage paw edema inhibition than that of *F. angolensis* methanol extract (53.21% vs. 40.82%, $P<0.05$) after 1 hour. This significant difference persisted up to 5 hours post edema induction (92.77% vs. 85.66%, $P<0.05$, **Table 1**). In contrast, *F. hildebrandtii* water extract at 250mg/kg, exhibited significantly lower paw edema inhibition compared to *F. angolensis* water extract, after one hour (43.45% vs. 59.6% $P<0.05$, **Table 1**), but significantly higher percentage paw edema inhibition than *F. angolensis* water at 5 hours post edema induction (75.44% vs.72.97%, $P<0.05$, **Table 1**). Notably, the two plant extracts did not exhibit any significant differences at lower tested doses. Altogether, these results show that at higher doses and after longer than 1 hour duration, *F. hildebrandtii* extracts had better activity in reducing paw edema than *F. angolensis* extracts.

Table 1

Comparison of Anti-Inflammatory Activities of F. hildebrandtii and F. angolensis Methanol and Water Extracts

Plant extract	Dose (mg/kg)	1st hr	2nd hr	3rd hr	4th hr	5th hr
F.H.W	250	**43.45±12.53$_{C}$**	40.13±10.58$_{DE}$	54.48±14.59$_{CD}$	72.00±5.87$_{B}$	**75.44±13.85$_{B}$**
F.A.W	250	**59.6±20.09$_{AB}$**	72.53±13.60$_{A}$	77.36±11.72$_{A}$	81.44±11.44$_{AB}$	**72.97±7.98$_{BC}$**
F.H.M	250	**53.21±7.15$_{BC}$**	60.35±7.55$_{B}$	74.48±8.48$_{AB}$	87.84±4.24$_{A}$	**92.778±1.925$_{A}$**
F.A.M	250	**40.82±18.48$_{C}$**	66.08±16.27$_{BC}$	73.99±9.21$_{AB}$	82.41±8.37$_{A}$	**85.66±6.04$_{AB}$**
F.H.W	50	38.3±23.9$_{D}$	34.6±33.6$_{F}$	52.5±26.9$_{D}$	61.39±19.68$_{C}$	67.44±17.03$_{C}$
F.A.W	50	56.08±51.6$_{B}$	60.41±8.87$_{B}$	68.07±3.30$_{B}$	80.90±19.67$_{AB}$	71.60±22.50$_{BC}$
F.H.M	50	52.49±8.37$_{B}$	50.26±16.89$_{CD}$	55.0±22.5$_{CD}$	63.31±16.12$_{C}$	60.11±18.82$_{D}$
F.A.M	50	40.68±16.28$_{D}$	58.83±5.89$_{B}$	71.09±3.53$_{AB}$	84.46±7.01$_{A}$	87.42±7.48$_{A}$
F.H.M	10	52.32±9.10$_{BC}$	59.07±11.03$_{B}$	71.90±8.32$_{AB}$	78..96±12.91$_{AB}$	86.11±8.11$_{A}$
F.A.M	10	36.36±2.37$_{C}$	44.57±10.64$_{D}$	50.8±26.3$_{D}$	71.36±21.78$_{B}$	67.21±16.49$_{C}$
F.H.W	10	37.86±19.79$_{D}$	32.4±32.7$_{F}$	43.7±24.5$_{E}$	61.72±15.93$_{C}$	65.00±18.48$_{C}$
F.A.W	10	43.41±7.31$_{C}$	59.52±6.72$_{B}$	63.45±9.18$_{C}$	79.54±7.59$_{AB}$	75.82±14.72$_{AB}$
F.H.W	2	35.9±28.8$_{E}$	37.6±30.9$_{E}$	52.29±20.34$_{D}$	63.5±24.0$_{C}$	60.1±23.9$_{D}$
F.A.W	2	39.0±29.4$_{D}$	47.70±14.56$_{D}$	62.33±9.38$_{C}$	66.82±4.36$_{C}$	72.67±9.09$_{BC}$
F.H.M	2	51.04±12.29$_{BC}$	53.10±13.40$_{C}$	62.29±15.83$_{C}$	73.38±3.04$_{B}$	71.89±5.02$_{BC}$
F.A.M	2	33.62±20.29$_{E}$	46.39±16.62$_{D}$	69.8±24.0$_{B}$	77.35±10.42$_{B}$	78.30±13.70$_{A}$

Analysis was done by One-way ANOVA, with statistical significance set at $P<0.05$. Similar subscript letters along the column denote none significantly differences. F.H.W- *F. hildebrandtii* water extract; F.A.W-*F. angolensis* water extract; F.H.M-*F. hildebrandtii* methanol extract; F.A.M-*F. angolensis* methanol extract. Statistically significant differences are highlighted in bold.

4.2 Comparative Analysis of Analgesic Properties

To study analgesic activity of the two plants, percentage writhing activity, which is a surrogate measure of pain was measured 15 minutes after oral administration of increasing concentration of the extracts (2-250 mg/kg) and compared using One-way ANOVA. As shown in **Table 2,** the percentage writhing inhibition of *F. hildebrandtii* methanolic extract was significantly lower than that of *F. angolensis* at 250 mg/kg (53.10% vs. 81.95 %, $P<0.05$). However, the water extracts of *F. angolensis* and *F. hildebrandtii* did not exhibit any differences at 250 mg/kg (83.80±6.73 vs 80.24±12.59, $P>0.05$). Comparative analysis at 50 mg/kg indicated that, methanolic extract of *F. angolensis* had significantly greater percentage writhing inhibition activity compared to *F. hildebrandtii* methanol extract (73.30% vs. 36.50%, $P<0.05$, One-way ANOVA). The percentage writhing inhibition of *F. angolensis* water extract at similar concentration was significantly lower than *F. hildebrandtii* water extract (70.30% vs. 77.76%, $P<0.05$, One-way ANOVA). At the lowest tested concentration of 2 mg/kg, the percentage writhing inhibition of *F. hildebrandtii* methanolic extract was significantly lower than that of *F. angolensis* methanolic extract (21.07% vs. 58.75%, $P<0.05$). There was no significant difference in percentage writhing inhibition between *F. hildebrandtii* and *F. angolensis* water extracts at 2 mg/kg (56.20% vs. 56.70%, $P>0.0.5$). Taken together, these results indicate that *F. angolensis* has better analgesic activity compared to *F. hildebrandtii* at both lower and higher concentrations. The standard aspirin 150 mg/kg had similar activity to both *F. angolensis* extracts at high concentrations ($P>0.05$).

Table 2

Comparison of Percentage Writhing Inhibition by F. hildebrandtii and F. angolensis Methanol and Water Extracts

Dose (mg/kg bw)	*F.angolensis* Water extract	*F.angolensis* Methanol extract	*F.hildebrandtii* Water extract	*F.hildebrandtii* methanolic extract	Aspirin	P value
250	83.80±6.73$_{A}$	81.95±10.75$_{A}$	80.24±12.59$_{A}$	53.10±6.10$_{C}$	84.99±3.26$_{A}$	$P<0.0001$
50	70.30±12.39$_{B}$	73.30±19.85$_{B}$	77.76±19.64$_{AB}$	36.50±8.05$_{C}$	84.99±3.26$_{A}$	$P<0.0001$
10	61.46±7.31$_{B}$	62.20±16.16$_{B}$	65.57±6.98$_{B}$	27.57±16.9$_{C}$	84.99±3.26$_{A}$	$P<0.0001$
2	56.70±31.40$_{B}$	58.75±19.71$_{B}$	56.20±17.76$_{B}$	21.07±11.77$_{C}$	84.99±3.26$_{A}$	$P<0.0001$

The same letters of the subscript within the rows are not significantly different from each other. Analysis was done by One-way ANOVA, at $P< 0.05$.

4.3 Comparative Analyses of Antioxidant Properties

As shown in Table 3 below, the percentage free radical scavenging activities of *F. hildebrandtii* and *F. angolensis* extracts were similar at lowest tested concentration of 0.01 µg/ml extract ($P>0.05$), but significantly higher than that of the standard ascorbic acid ($P<0.05$). Similarly, the percentage free radical scavenging activities of *F. hildebrandtii* and *F. angolensis* extracts were similar at maximum tested concentration of 1000 µg/ml ($P>0.05$, Table 3). These results show that antioxidant activities of the two different plant parts were equivalent at low and higher concentration. However, at concentration of 10 µg/ml, % RSA of *F. angolensis* water extract was significantly higher than % RSA of *F. hildebrandtii* water extract (68.10% vs. 54.82%, $P<0.002$). Similarly, at concentration of 100 µg/ml, *F. angolensis* water extract exhibited significantly higher % RSA than *F. hildebrandtii* water extract (84.06% vs. 66.83%, $P<0.0001$). Methanolic extract of *F. angolensis* at both 10 µg/ml and 100 µg/ml exhibited significantly greater % RSA than its *F. hildebrandtii* counterpart (70.47% vs. 54.54%, 90.13% vs. 72.88%, One-way ANOVA). Compared to the standard, the activity of both plant extracts had significantly higher activity compared to ascorbic acid at minimum concentrations while at maximum concentrations, there was no significant difference in activity between both plant extracts and standard, ascorbic acid 1mg/ml.

Table 3

Comparison of Free Radical Scavenging Activities of F. hildebrandtii and F. angolensis methanol and Water Extracts

Concentration (µg/ml)	*F.angolensis* Water extract	*F.angolensis* Methanol extract	*F.hildebrandtii* Water extract	*F.hildebrandtii* methanolic extract	L-ascorbic acid	P value
0.01	$53.25\pm7.58_A$	$47.94\pm3.85_A$	$48.81\pm5.59_A$	$49.26\pm3.63_A$	$26.51\pm6.77_B$	$P<0.036$
0.1	$54.84\pm7.60_A$	$49.67\pm3.65_A$	$52.56\pm7.21_A$	$49.57\pm3.65_A$	$30.94\pm2.18_B$	$P<0.053$
1	$56.14\pm8.27_A$	$52.67\pm3.62_A$	$55.17\pm5.93_A$	$50.62\pm3.23_A$	$33.82\pm2.94_B$	$P<0.026$
10	$68.10\pm4.82_B$	$70.47\pm2.04_B$	$54.82\pm6.85_C$	$54.54\pm3.23_C$	$88.12\pm6.49_A$	$P<0.002$
100	$84.06\pm2,36_A$	$90.13\pm0.59_A$	$66.83\pm5.6_C$	$72.88\pm1.87_B$	$95.80\pm0.24_A$	$P<0.0001$
1000	$86.56\pm3.25_A$	$91.85\pm0.56_A$	$93.64\pm1.39_A$	$93.56\pm0.90_A$	$96.38\pm0.89_A$	$P<0.030$
IC_{50}	0.01	0.9	0.98	0.987	5.674	

The same letters of the subscript within the rows are not significantly different from each other. Analysis was done by One-way ANOVA, at $P< 0.05$.

4.4 Discussion

Plants of the genus *Fagaropsis* are widely used traditionally in Africa to treat various disorders associated with inflammation, pain and oxidative stress (Mutinda et al., 2022). Although still limited, laboratory studies have confirmed that various plant parts of the species, *F. angolensis* and *F. hildebrandtii* indeed have potent anti-inflammatory and antioxidant activities (Mutinda et al., 2022). In the current study, we have compared the ant-inflammatory, analgesic and antioxidant activities of *F. angolensis* and *F. hildebrandtii*, two of the Fagaropsis *spp. that* are widely used as medicinal herbs in the Eastern Africa region. The findings suggest existence of some differences between the activities of *F. angolensis* and *F. hildebrandtii*, which depends on the extraction solvent and concentrations used.

The results indicate that both *F. angolensis* methanolic and water extracts at concentrations of 50 mg/kg and 250 mg/kg inhibited paw edema at all the experimental time points (1-5 hours) similar to the 10 mg/kg of dexamethasone standard control. However, at lower concentrations, the activities of *F. angolensis* methanolic and water extracts were inferior compared to dexamethasone. Similarly, the Carrageenan-induced paw edema was inhibited by *F. hildebrandtii* stem bark extracts as effective as 10 mg/kg of dexamethasone standard over the five-hour experimentation period, but to a lower extent at 2 mg/kg dose. These potent anti-inflammatory activities of *F. angolensis* leaves and *F. hildebradtii* stem bark extracts at high concentration in the current study are consistent with and extend the findings of a previous *in vitro* study of *F. angolensis* root bark by Mukavi et al. (2020). This potent anti-inflammatory activity is likely due to the presence of various compounds, including norphanes, steroids and phenols that have previously been detected in different parts of these plants (Mutinda et al., 2022). The quantity of these compounds might be low in diluted extracts, perhaps explaining the dose dependent on anti-inflammatory activity. The anti-inflammatory effect of dexamethasone is linked to inhibition of cyclooxygenases (COX-1 and COX-2) and consequent reduction of prostanoids and oxidative stress (Monteiro & Steagall, 2019). Accordingly, *F. hildebrandtii* and *F. angolensis* extracts might inhibit inflammation via similar mechanism of inhibiting production of prostanoids, however, this requires future detailed mechanistic studies.

Notably, comparative analysis showed that the anti-inflammatory activity of *F. hildebrandtii* methanol extract was superior to that exhibited by *F. angolensis* methanol extract. By contrast, the anti-inflammatory activity of *F.*

hildebrandtii water extract at 250mg/kg was lower compared to *F. angolensis* water extract, after one hour, but significantly higher at 5 hours post edema induction (**Table 1**). Since the anti-inflammatory activity is linked to the phytochemicals present in the extracts, the observed differences can be due to several factors. First, the *F. hildebrandtii* extracts was from the stem bark, while the extracts of *F. angolensis* were from the leaves. Although plants from the same genus generally have more less the same phytochemical profiles subtle, quantitative and qualitative differences can exist between the different plant parts. Therefore, it is possible that *F. hildebrandtii* stem bark methanolic extract had higher or qualitatively different anti-inflammatory phytochemicals compared to *F. angolensis* leaf methanol extract. Secondly, *F. hildebrandtii* samples were collected from Makueni whereas *F. angolensis* leaves were collected from Embu County. The difference in terms of climatic conditions and soil characteristics between these two regions can contribute to differential phytochemical profiles of the plant parts collected. It is also possible that methanol and water has different extraction efficiency of the bioactive compounds present in the two-plant part, with methanol extracting anti-inflammatory compounds more efficiently from *F. hildebrandtii* stem bark and water more efficiently from *F. angolensis* leaves.

The results of table 2 shows that at doses above 50mg/kg *F. angolensis* and *F. hildebrandtii* methanol extracts were as effective as 150 mg/kg of aspirin in relieving acetic acid-induced pain in mice, indicating good analgesic effects. Similarly, the water extracts of the two plants at concentrations above 50 mg/kg also exhibited analgesic effects that were comparable to that of 150 mg/kg of aspirin. Importantly, the methanol extracts of *F. angolensis* and *F. hildebrandtii* exhibited higher writhing inhibition compared to the water extracts at all the dose levels. This observation suggested higher extraction efficiency of analgesic compounds by methanol. Inflammatory mediators including prostaglandins, histamine, bradykinin and serotonin mediate the pain associated with writhing response observed in the animal model used in this study (Gawade, 2012; Lima et al., 2007). Therefore, the anti-inflammatory effect of *F. angolensis* and *F. hildebrandtii* extracts is most likely responsible for relieving of the acetic acid-induced pain. However, unlike the anti-inflammatory effects, the observed analgesic effects were dose-dependent, suggesting potential additional and direct anti-nociceptive mechanisms. Such putative mechanisms should be explored in future research. Furthermore, these results showed that *F. angolensis* and *F. hildebrandtii* could be similar to the conventional non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin which have both analgesics and anti-inflammatory effects (Slater et al., 2010). The results of this study, on *Fagaropsis* species are in agreement with similar research done on plants from the same family activities (Lima et al., 2007). It is also in line with the findings of other work that have demonstrated dose-dependent analgesic activity of herbal plants (Yimer et al., 2020).

Comparative analysis revealed that *F. hildebrandtii* methanolic extract had significantly lower analgesic activities than that of *F. angolensis* at all the tested concentrations (**Table 2**). However, this difference was not observed with the water extracts at any concentration. Taken together, these results indicate that *F. angolensis* methanolic leaf extracts has better analgesic activity in comparison with to *F. hildebrandtii* stem bark extracts. The findings suggest that for conditions that are associated with pain without substantial inflammation, *F. angolensis* methanolic extracts would be a better traditional treatment option. While it was not investigated in the current study, it is possible that specific compounds with unique analgesic properties might have been extracted more efficiently by methanol from *F. angolensis* leaves, hence the higher potency.

The *F. angolensis* and *F. hildebrandtii* water and methanol extracts exhibited dose dependent *in vitro* antioxidant activity as demonstrated by increasing % RSA **(Table 3).** Importantly, the plant extracts exhibited higher potency than ascorbic acid standard as highlighted by observed lower IC_{50} (**Table 3**). This data is not consistent with that of Alemu and Misganaw (2020), which demonstrated lower antioxidant activity of *F. angolensis* stem bark methanol extract compared to ascorbic acid. This discrepancy is likely to be due to the differences in the extraction methods used, as well as potential qualitative and quantitative differences in antioxidant phytochemicals of the leaves in this study versus the stem bark in their study. Oxidative stress is a major factor in the pathogenesis of inflammation and pain (Arulselvan et al., 2016; Farmer, 2020; Sommer et al., 2018). Therefore, the potent free radical scavenging partly explains the observed analgesic and anti-inflammatory properties of the different plant part extracts. Comparative analysis showed some differences in the antioxidant activities of the different plant part extracts at medium tested concentration. Specifically, *F. angolensis* water extract at 10 µg/ml and 100 µg/ml exhibited high antioxidant in comparison to *F. hildebrandtii* water extract. This indicates superior antioxidant activity of *F. angolensis* leaf water extract.

V. CONCLUSION & RECOMMENDATIONS

5.1 Conclusion

In conclusion, water and methanol extracts of leaves and barks of Fagaropsis spp. have potent antioxidant, analgesic and anti-inflammatory effects. This validates the widespread use of this plant in the treatment of diseases

associated with inflammation, pain and oxidative stress. However, there are subtle differences in the anti-inflammatory, antioxidant and analgesic activities of the different plant parts of the genus *Fagaropsis* when extracted using difference solvents. *F. angolensis* leaf water extract has higher antioxidant activity in comparison to *F. hildebrandtii* water extract. *F. angolensis* methanolic leaf extracts has better analgesic activity in comparison with *F. hildebrandtii* stem bark extracts. *F. hildebrandtii* stem bark methanol extract exhibits superior anti-inflammatory activity compared to *F. angolensis* leaf methanol extract.

5.2 Recommendations

Researchers recommend conservation of *F. angolensis* and *F. hildebrandtii* and further studies to isolate and characterize the potential bioactive compounds of these plants. Because of the observed differences, there is need to use specific plant parts for specific conditions depending on whether there is co-existence of both pain and inflammation.

Conflict of interest

The authors declare no conflict of interest.

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