Scientific African 23 (2024) e02088

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Phytoconstituents of Kenyan stinging nettle (*Urtica* species) and their molecular docking interactions revealed anti-inflammatory potential as cyclooxygenase-2 inhibitors

Jacqueline Wambui [a,*], Robert I.O. Ikedi [b], Rosaline W. Macharia [a], Francisca Kama-Kama [c], Evans N. Nyaboga [a]

[a] Department of Biochemistry, Faculty of Science and Technology, University of Nairobi, P.O. Box 30197 - 00100, Nairobi, Kenya
[b] Department of Physics, Faculty of Science and Technology, University of Nairobi, P.O. Box 30197 - 00100, Nairobi, Kenya
[c] Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000 - 00200, Nairobi, Kenya

ARTICLE INFO

Editor: DR B Gyampoh

Keywords:
Phytochemicals
Anti-inflammation
Stinging nettle
Urtica sp

ABSTRACT

Inflammation is a complex, natural protective response towards different stimuli characterized by the dilation and permeation of the blood vessels with a surge in leukocytes in the tissues. The current treatment involves the use of anti-inflammatory drugs, corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs), which have been associated with adverse side effects especially gastrointestinal ulcers. Therefore, there is a growing need to explore alternative sources from medicinal plants. In the present study we investigated anti-inflammatory activities of the leaves of the Kenyan stinging nettle using *in vivo* and *in silico* molecular docking. Molecular identification of plant sample was carried out based on DNA barcoding. Crude extracts were prepared using water and methanol: dichloromethane (1:1) and preliminary identification of total phenolic and flavonoids was carried out using Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively. The classical model of Carrageenan- induced paw edema was used to test the *in vivo* anti-inflammatory activity of the extracts. The extract was screened using Laser Raman Spectroscopy and Liquid Chromatography Mass Spectroscopy (LC-MS) and the molecular interaction between the identified compounds within the binding site of Cyclooxygenase-2 (COX-2) performed through molecular docking as the confirmatory tool of *in vivo* experiments. Based on DNA barcoding analysis, the plant sample was identified as *Urtica* species. The total phenolic content of the aqueous and methanol: dichloromethane extracts were 3.75 mg gallic acid equivalents (GAE)/g dry sample and 6.26 mg GAE/g dry sample while total flavonoid content were 0.3872 mg quercetin/g dry sample and 1.76 mg quercetin/g dry sample, respectively. The aqueous extract significantly ($p < 0.05$) reduced the paw edema in the Carrageenan model of inflammation. LC-MS confirmed the presence of 19 phytochemicals, of which 10 and 9 were phenolic and flavonoid compounds, respectively. From these identified compounds quercetin attained the lowest binding energy when complexed with COX-2, followed by rhamnetin, quercetin rhamnoside, epigallocatechin gallate and chlorogenic acid. Molecular docking studies supported the *in vivo* findings and confirmed the anti-inflammatory potential of *Urtica* sp. These findings suggest *Urtica* sp. is a potential source of bioactive compounds that could be employed in

* Corresponding author.
E-mail address: jewjackie@gmail.com (J. Wambui).

https://doi.org/10.1016/j.sciaf.2024.e02088
Received 2 October 2023; Received in revised form 5 January 2024; Accepted 11 January 2024
Available online 14 January 2024

the future development of anti-inflammation drugs. Further studies can attempt to assess the individual isolated active compound(s) for their anti-inflammatory activity.

Introduction

Inflammation is an immunological defense mechanism that is triggered in response to viral, bacterial or parasitic attacks, burn allergens, mechanical injuries and other noxious stimuli. Studies have reiterated the role of phytochemicals from medicinal plants in modulating inflammation [1]. Some medicinal plants have anti-inflammatory properties and have been used as pain relievers in alternative medicine [2,3]. Others are capable of eliciting pharmacological responses like non-steroidal anti-inflammatory drugs (NSAIDs) by inhibiting cyclooxygenase (COX) [2]. NSAIDs and corticosteroids are among the conventional treatment strategies used for inflammation [4]. However, prolonged use of NSAIDs has been associated with gastro- intestinal, renal and cardio complications and development of drug allergies [5,6]. Also, corticosteroid long term use has been linked with ophthalmic, gastrointestinal and hepatic-related complications [7]. The development of new and less toxic anti-inflammatory drugs is now a growing concern worldwide. Consequently, naturally occurring alternatives from medicinal herbs and plants can be explored to avert the negative sequels of conventional medicine.

Plant-based bioactive compounds have been applied in integrative medicine, whose scope focuses on conventional and non-conventional methods and utilizes indicators such as environment, food, and health, which collectively form the wheel of health [8]. One of the promising medicinal plants to treat inflammation is stinging nettle (Urticaceae family). In folk medicine, the leaves, stems and roots have been used to treat rheumatism, arthritis or to promote anti-proliferation, hypotensive and anti-inflammatory effects [9,10]. Previous research has shown that stinging nettle can also be used alongside NSAIDs [9]. In particular, the stinging nettle, *Urtica dioica*, is believed to contain anti-inflammatory phytochemicals that have been reported to alleviate pain in osteo-arthritis patients [10]. Phytalgic™ is a nutraceutical product for osteoarthritic patients, made up of fish oils, vitamin E, *Urtica dioica* and zinc [11]. Moreover, the hexane extracts of the stinging nettle leaves of *Urtica dioica* were found to have anti-inflammatory properties while using anti-inflammatory drug indomethacin, as the control in paw edema test [12]. Although previous researches have studied the anti-inflammatory ability of the stinging nettle *Urtica dioica*, there is limited data on the potential of *Urtica* sp. from Kenya as source of anti-inflammatory compounds. Studies have been conducted on *Urtica* species from other African Counties and documented its anti-inflammatory activities [9,10]. However, anti-inflammatory properties of *Urtica* species from Kenya have not been documented. In Kenya, stinging nettle has been known for a long time in traditional medicine for the treatment of many diseases. This study was based on reports of traditional use, aiming to fill unexplored gaps in scientific research on the anti-inflammatory action of the species. Nevertheless, previous research on the ethnobotanical uses of *Urtica* species showed that the leaves of this plant have been used by Kenyan communities for the treatment of malaria, skin and stomach infections [13,14]. Unearthing its chemical composition and anti-inflammatory potential offers a basic understanding of biological implications to human health.

Different studies have used different experimental animal models to evaluate the *in vivo* anti-inflammatory activity of natural and synthetic compounds. Carrageenan-induced paw edema is one of the most widely used models for screening anti-inflammatory activity of plant-based remedies [15]. It is a classical animal model for evaluation of acute anti-inflammatory agents [15,16]. Carrageenan is an effective substance that promotes the production and release of inflammatory and pro-inflammatory mediators such as pro-cytokine, histamine and prostaglandin formed by cyclooxygenases [15,16]. It is highly sensitive and reproducible test for nonsteroidal anti-inflammatory drugs and has been established as a valid model to study new anti-inflammatory compounds and drugs. Carrageenan model is typically linked with the activation of the cyclooxygenase pathway. Therefore, this model is used to screen natural non-steroidal anti-inflammatory molecules that are cyclooxygenase inhibitors [17,18].

The aims of the present study were to: (i) screen for phytochemicals in crude extracts of *Urtica* sp. leaves; (ii) analyze the anti-inflammatory activity of *Urtica* sp. crude extracts using *in vivo* method and (iii) determine the anti-inflammatory activity of the bioactive compounds present in the *Urtica sp*. crude extracts using in silico approach. This might open the door to the discovery of bioactive chemical substances without the negative side effects usually connected with synthetic drugs.

Materials and methods

Sample collection

Plant material, mainly leaves were collected from Limuru, Central Kenya (1.1069° S, 36.6431° E). The study site, Limuru, Central Kenya, is 2500 m in altitude and has cool temperatures of 10–28 °C where stinging nettle grows natively. Verbal consent was sought from the subsistence farmer prior to plant samples collection. The leaves packed into khaki bags and transported to the Faculty of Science and Technology, University of Nairobi, where a voucher specimen was deposited in the herbarium unit in the Department of Biology with a reference voucher number JW/UON2021/001. Later, the plant leaves were air dried at room temperature, away from the sunlight and after which they were ground into fine powder using a mortar and pestle and stored -4 °C for further use [19].

Genomic DNA extraction and PCR amplification

Genomic DNA was extracted from approximately 200 mg fresh leaf samples using Cetyltrimethylammonium bromide (CTAB)

protocol [20]. Polymerase chain reaction (PCR) amplification was carried out in a reaction volume of 25 µl consisting of 2.5 µl 10 × PCR buffer, 1.0 µl magnesium chloride, 1.0 µl dNTP mixture, 0.5 µl each primer, 0.125 µl Taq polymerase, and 2 µl of 50 ng DNA template and 17.375 µl double distilled water. The thermal cycling conditions used for the ribulose 1, 5-bisphosphate carboxylase/oxygenase (*rbcL*) and internal transcribed spacer (ITS) primers (Supplementary Table S1) were: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for *rbcL* (and 55 °C for ITS) for 1 min and extension at 72 °C for 80 s with a final extension at 72 °C for 10 min. The products of PCR were checked on 1% (w/v) agarose gels, followed by purification and then sent to Inqaba Biotec East Africa Ltd for Sanger sequencing.

Sequence and phylogenetic analysis

The forward and reverse sequences read (.abi) files, were converted to a .txt (text) file and then concatenated. A contig sequence was developed from combined file using the Cap Contig Assembly program in BioEdit® version 7.2.5.0 [21]. The contig sequence was then subjected to a homology search using BLASTN under the default parameters [22]. A similarity search for each sequence was verified using BLASTn program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the identity of the sequences. Phylogenetic trees were constructed based on Bayesian inference (BI) method using MrBayes version 3.2.7 (https://nbisweden.github.io/MrBayes/; accessed on 16th January 2023). The constructed phylogenetic trees were visualized and modified by FigTree software version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/; accessed on 17th January 2023).

The identification of the plant sample based on the *rbcL* and ITS sequences was evaluated by the BLAST and phylogenetic tree-based methods. Each of the sequence was aligned in the NBI database by BLAST sequence similarity search and the best match was selected as the identification results with E value < cut-off value (E value < 10^{-6}). Based on the phylogenetic tree method, when all individuals of the same species can be clustered in a single clade, the species is considered to be successfully identified.

Crude extraction

We carried out the extraction procedure using two solvent systems, namely a mixture of methanol and dichloromethane (1:1) and water using the following protocol: Hundred grams of the powdered sample were soaked into a mixture of methanol: dichloromethane into a ratio of one to one. Extraction procedure was carried out three times using cold percolation methods. Residues were separated from the filtrate using a filter paper whereby the residues were used to carry out the second and the third extractions. The filtrate from the three extractions were concentrated using a rotary evaporator at (50–55 °C), cooled at room temperature and stored at 4 °C for further uses [23,24].

For the aqueous extract, 50 g of the powdered sample was soaked into 1000 ml of distilled water. The mixture was boiled using a hot plate for 15 min, cooled, and filtered [19,25]. The filtrate was cooled at room temperature, stored in a deep freezer before lyophilization after which it was kept at 4 °C for further analysis [21].

Estimation of total phenolic content

Estimation of the phenolic content was done by reconstituting the aqueous and methanol: dichloromethane (1:1) extracts (1 mg) each in 1 ml of methanol to make a solution of 1 mg/ml. An a liquot sample of 1 ml was introduced into a 10 ml volumetric flask and was mixed with 2.5 ml of Folin-Ciocalteu (FC) reagent (1:10 diluted with distilled water) followed by the addition of 2 ml of 7.5% sodium carbonate. The assay mixture was topped up to the mark using distilled water. The mixture was incubated at 45 °C for 15 min, and absorbance was measured at 765 nm. The total phenolic content (mg/ml) was calculated using gallic acid as standard [26]. Total phenolic content was calculated using the formula, (TPC) = C (V/M), where C = concentration of gallic acid (mg/ml), Dilution factor = V/M, V = volume of extract and, M= mass of extract. Total phenolic content (TPC) was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg GAE / g).

Estimation of total flavonoid content

The aqueous and methanol: dichloromethane extracts (1 mg) were each prepared in 1 ml of methanol (1 mg/ml). An aliquot sample of 1 ml was put into a 10 ml volumetric flask where it was mixed with 4 ml of distilled water followed by the addition of 0.3 ml of 5% sodium nitrite. The assay mixture was allowed to rest for 5 min after which 0.3 ml of 1% aluminum chloride was added. The resulting mixture stood for another 5 min, followed by the addition of 2 ml, 1 M sodium hydroxide. The mixture was then topped up to the mark using distilled water. Absorbance was measured at 510 nm. The total flavonoid content (mg/ml) was calculated using quercetin as standard [26]. Total flavonoid content was calculated using the formula, TFC = C (V/M), where C = concentration of quercetin (mg/ml), Dilution factor =V/M, V = volume of extract and M = mass of extract. Total flavonoid content (TFC) was expressed as mg quercetin per gram of sample in dry weight (quercetin mg/g).

Raman spectroscopic analysis of the crude extracts

STR-series Raman spectrometer (Technos Instruments) equipped with a 785 nm Near Infrared diode laser, a thermoelectric cooled CCD detector with 2048 pixels and manually controlled microscope stage was used for analysis. Instrumental parameters were set as follows: density filter was set to allow 50% of laser power to pass to the sample (with a maximum power output of 18.20 mw and a spot

size of 68.47 mm), × 10 (0.30) objective lens was used to focus the sample under study at the microscope stage, acquisition time was 10 s, 5 accumulations per sample spot and spectra was acquired in the region of 97–1846 cm^{-1} at a resolution of 1.78. The 600 BLZ grating was used. Before starting the experiment, the Raman system was first cooled to -76 ℃ and calibrated using a silicon wafer peaked at 520.5 cm^{-1} and centered at 1050 cm^{-1}. All experimental measurements were done at room temperature in a dark room. Background correction, baseline subtraction, normalization and principal component analysis (PCA) were conducted using Chemo Spec [27] as the preprocessing steps. The cleaned dataset was visualized using OriginLab software and therein the peak wavelengths were identified [28].

In vivo anti-inflammatory activity

In vivo anti-inflammatory activity test was carried out using Carrageenan model. Prior to the experiment, ethical approval (FVM BAUEC/2021/314) was obtained from Biosafety, Animal use, and Ethics committee of the Faculty of Veterinary Medicine, Department of Veterinary Anatomy and Physiology. A total of 20 male and female albino rats weighing 180–250 g were randomly divided into four experimental groups with each having a sample size of five. Groups A and B received the aqueous and methanol: dichloromethane extracts each at 750 mg/kg body weight as described by Adeyemi et al. [29]. Group C, the reference/positive control, received both the carrageenan and indomethacin, an NSAID, at a dosage of 10 mg/kg [29]. Group D received 1% pure carrageenan treatment at 100 µl and saline water at 0.9% (w/v) (carrageenan/negative control) as described by Amdekar et al. [30].

The protocol for acute inflammation was adopted from the Carrageenan model. The aqueous and methanol: dichloromethane extracts were administered orally to Group A and B at 750 mg/kg body weight. This treatment was administered 30 min before injection of carrageenan into the sub-plantar region of the right hind paw of every rat [31]. Changes in volume were measured using a plethysmograph at 0, 0.5, 1, 1.5, 2, 2.5 and 3 h after carrageenan administration. An increase in paw volume is calculated as the difference between the "0″and at respective hours [31]. Paw volumes indicated is the difference in paw volume before and after administration of the extracts or controls. From the paw volume measurements, the percentage inhibition of edema was calculated as the difference between the initial and final paw volumes of the control group and test group recorded each at the same time [32].

The formula used for calculation of percentage inhibition of edema was% inhibition of edema $= (A-B)/A \times 100$, where $A =$ Paw volume of the control (negative) group and $B =$ Paw volume of the test group.

Statistical analysis

The differences between treatments for paw edema are expressed as mean, with ± as the standard deviation for a sample size of 5. Statistical analyses were performed using a one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Significant results were considered at a p-value < 0.05.

Reverse phase high pressure liquid chromatography mass spectrometry (RP LC-MS)

Air-dried powder sample of 100 g was processed in high pressure liquid chromatography (HPLC) grade methanol while being sonicated for two days and filtered. The filtrate was then evaporated using Buchi Rotary Evaporator in a water bath with vacuum of 600 Psi to achieve 3.5 g of the extract. The 1 g of the extract was weighed with Sartorius analytical balance and centrifuged at 15,000 rpm for 2 min (J2–21 MIE, JA-20 rotor, Beckman, UK) and using a glass pipette and transferred into small clean labeled vials. Centrifugation was undertaken to remove all particulates from the extracts, thus preventing the possibility of column blockages. From the extract, the stock solution was prepared of 1 mg/ml, and 1 ml of the stock solution was placed into an HPLC vial (pyrex) and 20 µl was injected into liquid chromatography mass spectroscopy (LC-MS) electrospray ionization (ESI) vial [33]. The LC-MS-ESI key working parameters were as follows: flow rate at 1 ml/minute, injection volume at 20.0 µL, dwelling time was 50 ms, capillary voltage, 3.0 kV, cone voltage, 70 V, extract voltage, 5 V; RF voltage, 0.5 V; source temperature, 110 C; nitrogen gas temperature for desolvation, 380 °C; and nitrogen gas flow for desolvation, 400 l/h. Using rutin as the standard (1–100 ng/µl) the linear calibration curve (peak area *vs*. concentration) obtained was [$y = 6008.9x - 5250.3$ ($R^2 = 0.9987$)] and it served as a basis for external quantification [34].

In silico molecular docking

With relation to the *in vivo* model of inflammation, the carrageenan induced paw edema expresses and regulates cyclooxygenase II (COX-2) [35]. COX-2 that produces PGs is closely associated with inflammatory disorders of acute as well as chronic types. COX-2 enzyme is a target for a wide range of many anti-inflammatory drugs as it is responsible for production of PGs inducing inflammation. In addition, COX-2 selective inhibitors possess anti-inflammatory activity with reduced side effects frequently seen with cyclooxygenase I (COX-1) non-selective inhibitors. Therefore, cyclooxygenase-2 enzyme, was selected as the protein receptor. Its crystallographic structure in the Protein Data Bank (4-COX) complexed with ligands: HEM (protoporphyrin IX containing Fe), IMN (indomethacin) and NAG (2-acetamido-2-deoxy-beta-D-glucopyranose). Ligand, indomethacin was used as the non-selective inhibitor [36]. Protein receptor was prepared for docking using BIOVIA Discovery Studio 2021 Client version 21.1.0. Ligands (HEM, NAG and IMN (indomethacin)) were removed followed by polar hydrogen atoms addition. Next, the grid box configuration was determined by obtaining the x, y and z configuration values of indomethacin. The resulting protein file was converted to pdbqt using Open Babel 2.4.0. The pdbqt file was loaded to Auto Dock Tools 4.2.6 whereby water molecules deletion, addition of Kollman charges and computation of Gasteiger charges operations were applied to the protein [37].

The ligands were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) and saved in Canonical SMILES format. The ligand was prepared by addition of hydrogen and charges using Gasteiger method using Chimera's (version 1.16) minimize structure command then conFig.d by Auto Dock Tools 4.2.6 to form a pdbqt file [37]. The final configuration parameters for docking using Autodock Vina (version 1.1.2) were: center_x = 14.949600, center_y = 52.163200 and center_z = 68.722120; size_x = 20, size_y = 20 and size_z = 20; exhaustiveness = 8 [38]. Based on Diallo and colleagues (2021), only docking values below 2 Angstrom in root mean square deviation (RMSD) were considered [39]. Structural analyses were done to the protein-ligand docked complexes to investigate the interaction forces and the subsequent amino acid residues involved.

Results

Molecular identification of the plant sample

To determine the identity of the plant samples, the percentage similarity and their corresponding E values of BLASTN search were used. According to the alignment results for the *rbcL* marker, *Urtica* sp. registered the highest identity of 99.13% with 0.0 E-values and 95% query coverage (Supplementary Table S2). Similarly *Urtica* sp. was found to be homologous to the plant sample with 93.48% similarity, 0.0 E-values and 92% query coverage (Supplementary Table S2). BLASTn analysis of the *rbcL* and ITS2 sequences identified the plant sample to belong to the genus *Urtica*.

Phylogenetic analysis of the *rbcL* and ITS2 sequences resulted in a well resolved phylogeny. A phylogenetic tree constructed using combined ITS2 and *rbcL* sequences yielded two major clusters that were robust with 93% bootstrap support values (Supplementary Fig. S1). The plant sample was identified as *Urtica* species.

Phytochemical composition of the crude extracts

The yield of crude extracts obtained from the aqueous extract (14%) was significantly higher than that of methanol: dichloromethane (9.6%) (Table 1). Preliminary phytochemical screening on total phenolic and total flavonoid contents were determined by Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively, for each crude extract. For phenolic content, the aqueous extract contained 3.75 ± 0.43 mg GAE/g dry sample while methanol: dichloromethane extract had 6.26 ± 0.276 mg GAE/g dry samples (Table 1). The quantitative determination used standard calibration curve of gallic acid (GAE) ($y = 8.81x$, $R^2 = 1$). With regard to the total flavonoid content, the methanol: dichloromethane (1.76 ± 0.315 mg quercetin/g dry sample) was nearly thrice that of aqueous (0.3872 mg quercetin/g dry sample). The quantitative determination used quercetin standard calibration curve ($y = 0.0065x + 0.0059$, $R^2 = 0.5234$).

Raman spectroscopic analysis of the crude extracts

The raw spectra for the methanol: dichloromethane and aqueous extracts were obtained at laser powers of 1–5% (Supplementary Figs. S2 and S3) and 50–100% (Supplementary Figs. S4 and S5), respectively. These raw spectra were pre-processed by correction for background fluorescence using Vancouver Raman Algorithm. The processed spectra of methanol: dichloromethane and aqueous extract spectra ranged from 97 to 1847 cm^{-1} while their optimum was obtained at 1250 cm^{-1} and 100 cm^{-1} wavelength, respectively (Fig. 1).

Baseline subtraction estimated the unknown background by polynomial baseline fitting, Savitzky- Golay smoothing coupled with first or second order differentiation (Fig. 2). Normalization adjusted the samples and experimental variables. Based on the principal component analysis (PCA), a positive loading showed a greater importance than a negative loading to the principal component.

The PCA analysis of PC1 (79%) and PC2 (14%) effectively differentiated between methanol: dichloromethane and aqueous extracts. The methanol: dichloromethane extract (DCM in green) dominated positive PC2 while negative PC2 was dominated by both methanol: dichloromethane extract and aqueous extract (WE in red). Both the methanol: dichloromethane and aqueous extracts were scattered along negative PC1 and PC2 while methanol: dichloromethane extract was found only in negative PC1 and positive PC2 (Fig. 3). The spectral range between 280–500, 800–1000 and 1000–1180 cm^{-1} as well as 1300–1350 cm^{-1} and 1550 – 1700 cm^{-1} distinguished between the methanol: dichloromethane and aqueous extracts (Fig. 4).

Phytochemicals identification based on Raman spectroscopic analysis

Spectral analysis of the aqueous extract using Origin software revealed bands at 733 cm^{-1} (Fig. 5). The bands are associated with Cycl. [ω(C=O)] vibrations in 3-caffeoylquinic acid, 5-caffeoylquinic acid, 3, 4-caffeoylquinic acid, and 4, 5-caffeoylquinic acid. Also,

Table 1
Percentage yield, total phenolic and flavonoid content for the methanol: dichloromethane and aqueous extracts of the stinging nettle.

Extract	Yield (%)	Total phenolic content (mg GAE/g)	Total flavonoid content (mg quercetin/g)
Aqueous	14	3.75 ± 0.43	0.3872
Methanol: dichloromethane	9.6	6.26 ± 0.276	1.76 ± 0.315

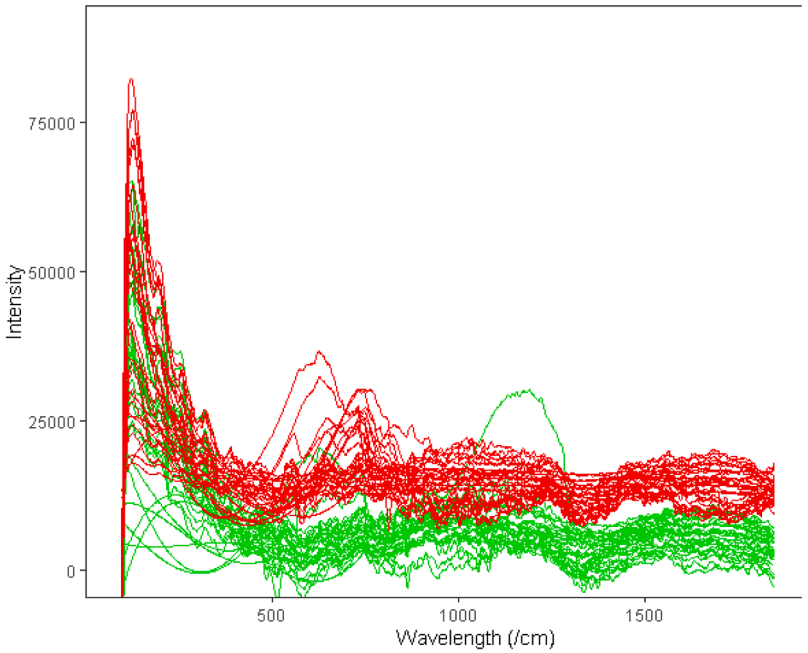

Fig. 1. Processed Raman spectra after background correction using Vancouver algorithm for the methanol: dichloromethane and aqueous extract of the stinging nettle. The methanol: dichloromethane extract is presented in green and aqueous extract in red.

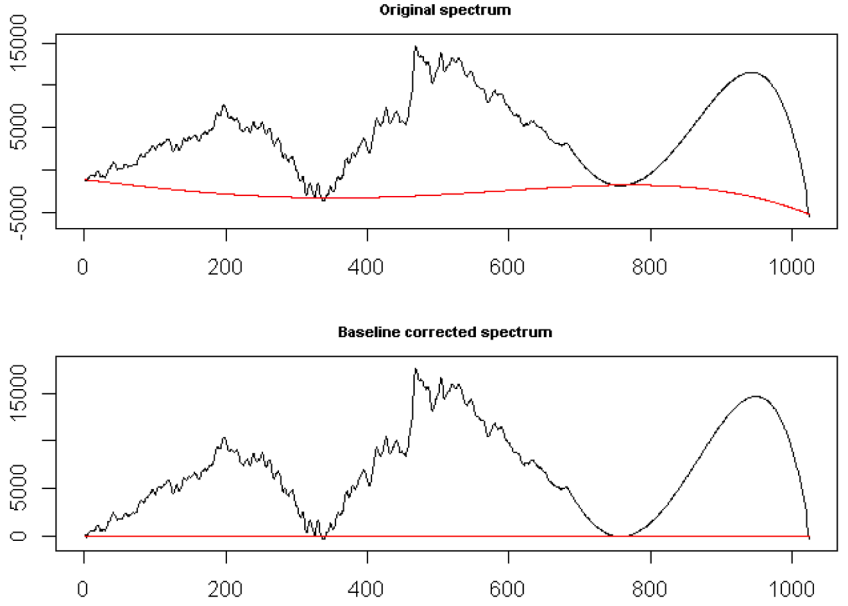

Fig. 2. Baseline subtraction for the methanol: dichloromethane (upper) and aqueous (lower) spectra of the stinging nettle.

epigallocatechin was identified at 733 cm^{-1}. The identified phytochemical compounds are presented in Table 2 and the respective bands in Fig. 5.

In vivo anti-inflammatory activity

The anti-inflammatory activity of the stinging nettle extracts was tested through the development of paw edema (Table 3). Paw volume of negative control animals was initially at 12.04 ± 0.483 and increased up to 15.08 ± 0.595 after 2.5 h. For positive control animals, the paw volume started at 9.52 ± 0.205. By the end of 2.5 h, the paw volume was at 13.08 ± 1.055. The paw volume of

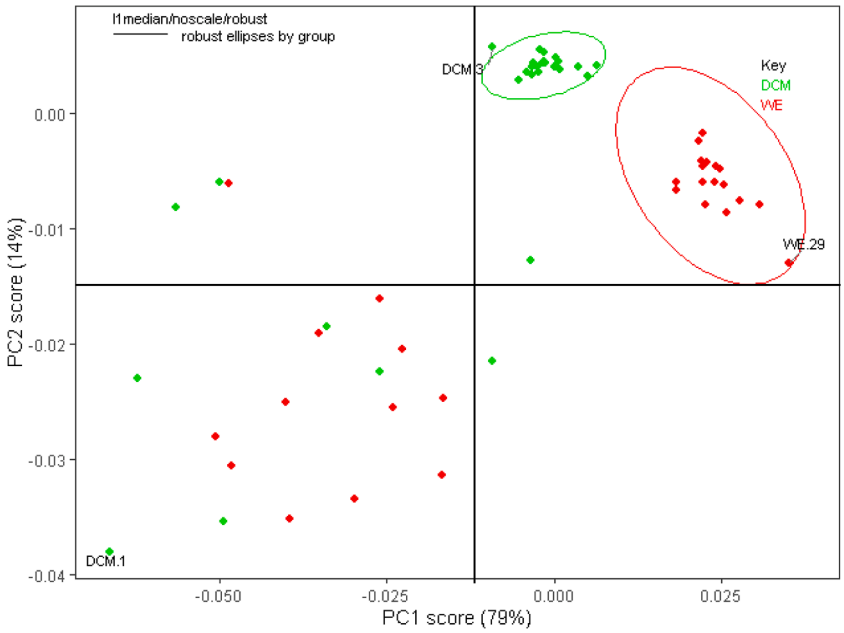

Fig. 3. PCA analysis for the aqueous extract and methanol: dichloromethane spectra of the stinging nettle with PC1 score at 79% while PC2 score at 14%. The methanol: dichloromethane (DCM) extract is presented in green and the aqueous (WE) extract in red.

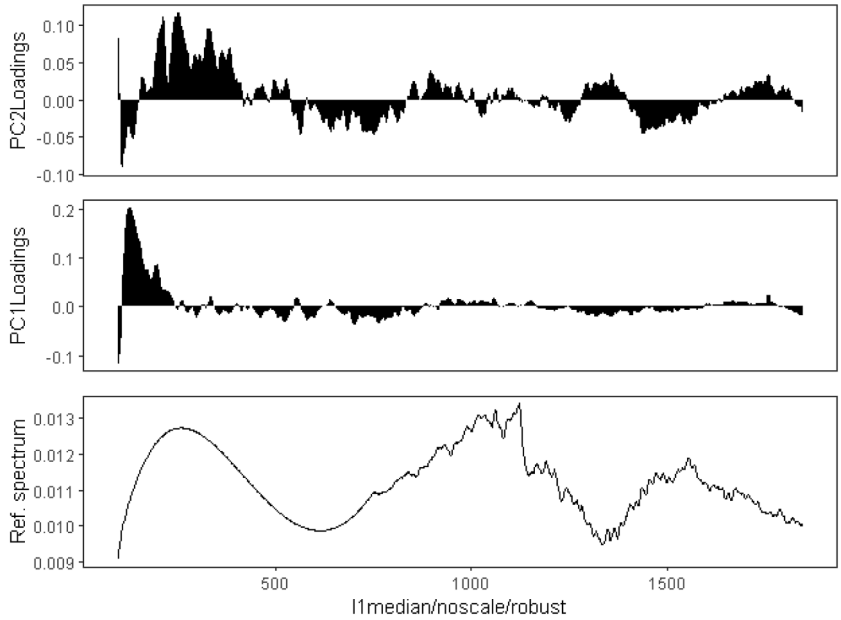

Fig. 4. PC loadings for both PC1 and PC2 from the PCA analysis for the methanol: dichloromethane and aqueous extracts of the stinging nettle. Positive loadings for both PC1 and PC2 are present mostly in spectra below 500 cm^{-1}.

animals treated with methanol: dichloromethane and aqueous extracts were 9.36 ± 0.285 and 9.2 ± 0.433, respectively, at zero time and 15.44 ± 0.655 and 12.6 ± 0.707 after 2.5 h respectively. The results demonstrated that the revealed the aqueous extract was significantly ($p<0.05$) lower than the negative control.

All experiments were carried out using 5 replicates. Values are expressed in paw volume values were expressed as mean, with ± as the standard deviation for a sample size of 5; *$p < 0.05$ significant comparison to the negative control. At $p < 0.05$, the aqueous extract was found to be statistically different from the negative control group.

The maximum percentage inhibition for the positive control was 17.28% after 2 h while the aqueous extract showed a maximum

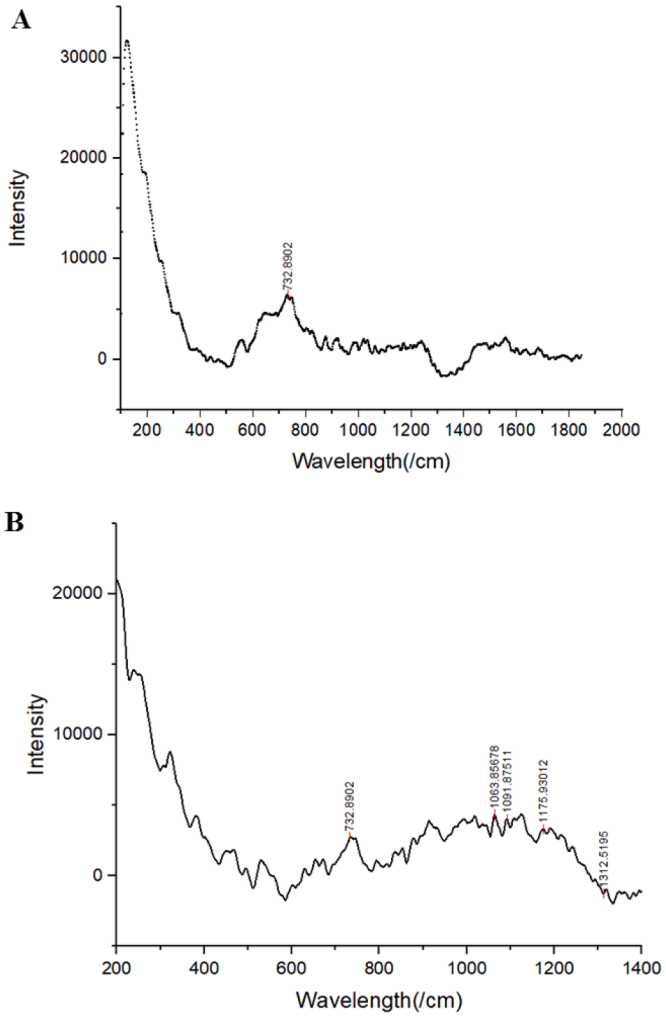

Fig. 5. Average Raman spectrum for the (A) aqueous extract of the stinging nettle with the identification of compounds at 733 cm^{-1}; and (B) methanol: dichloromethane extract of the stinging nettle with the identification of compounds at 733, 1064, 1092, 1176 and 1213 cm^{-1}.

inhibition of 22.35% after 1.5 h (Table 4 and Fig. 6).

For the methanol: dichloromethane extract, a maximum inhibition of 16.81% was attained after 30 min. Comparing the percentage inhibition rate, the aqueous extract was significantly ($p < 0.05$) higher than the methanol: dichloromethane extract and positive control (Table 4).

Reverse phase liquid chromatography mass spectroscopy (LC-MS)

Reverse phase-LC-MS fractionation and quantification of the *Urtica* sp. unraveled a cocktail of 19 substances for which the identity is detailed in Table 5. The phytochemical profile showed that the *Urtica* sp. extract is rich in phenolic acids and flavonoids. Out of the 19 substances identified, 10 were phenolic acids (cinnamic acid, p-coumaric acid / 4 hydroxycinnamic acid, caffeic acid, sinapic acid, ferulic acid, chlorogenic, gallic acid, syringic acid, gentisic acid/dihydroxybenzoic acid and protocatechuic acid), while 9 were flavonoids (quercetin, myricetin, rhamnetin, kaempferol hexoside or kaempferol 3-O hexoxyl hexoside, kaempferol-3-rutinoside, isorhamnetin rutinoside or isorhamnetin-3-O-rutinoside and epigallocatechin gallate). The major compounds identified were quercetin rhamnoside (40.64%), kaempferol hexoside (31.10%), isorhamnetin rutinoside (5.87%), p-coumaric acid (4.62%) and chlorogenic acid (4.55%) while minor compounds quercetin-3 glucoside (0.38%), rhamnetin (0.46%), syringic acid (0.54%), gentisic acid (0.58%) and gallic acid (0.59) (Fig. 7).

Structures of selected compounds identified from both Raman spectroscopy and LC-MS

Among the phytochemical compounds identified from the stinging nettle using both Raman spectroscopy and LC-MS were phenolic acids (gallic acid, 3-caffeoylquinic acid, cinnamic acid, p-coumaric acid, and chlorogenic acid) and flavonoids (quercetin, myricetin,

Table 2
Identification of compounds for the aqueous and methanol: dichloromethane extracts of the stinging nettle spectrum in terms of wavelength and vibrational assignment.

Extract	Wavelength (cm^{-1})	Vibrational mode	Assignment
Aqueous extract	733	Cycl.[ω(C=O)]	CaffeoylQuinic acid [40]
		Cycl.[ω(C=O)]	5-CaffeoylQuinic acid [40]
			Cycl. [ω(C=O)]
		Cycl. [ω(C=O)]	3,4-diCaffeoylQuinic acid [40]
		Cycl. [ω(C=O)]	3,5-diCaffeoylQuinic acid [40]
		Cycl. [ω(C=O)]	4,5-diCaffeoylQuinic acid [40]
		–	Epi-gallocatechin [41]
	1241	–	Ferulic [41]
Methanol: dichloromethane	733	Cycl. [ω(C=O)]	3-CaffeoylQuinic acid [40]
		Cycl. [ω(C=O)]	5-CaffeoylQuinic acid [40]
		Cycl. [ω(C=O)]	3,4-diCaffeoylQuinic acid [40]
		Cycl. [ω(C=O)]	3,5-diCaffeoylQuinic acid [40]
		Cycl. [ω(C=O)]	4,5- diCaffeoylQuinic acid [40]
		–	Epigallocatechin [41]
	1064	–	Genistein [41]
	1091	–	Tannic [41]
	1175	–	Quercetin [41]
	1313	–	4-Hydroxybenzoic [41]

Table 3
Paw volumes for aqueous, methanol: dichloromethane extracts of the stinging nettle and experiment controls.

	Paw volumes for different extracts					
Extract	Initial	½ h	1 h	1.5 h	2 h	2.5 h
Negative Control	12.04 ± 0.483	13.32 ± 0.215	13.06 ± 0.252	13.96 ± 0.354	15.04 ± 0.770	15.08 ± 0.595
Methanol: dichloromethane extract 750 mg/kg	9.36 ± 0.285	11.08 ± 0.344	12.96 ± 0.462	13.16 ± 0.457	14.64 ± 0.858	15.44 ± 0.655
*Aqueous extract 750 mg/kg	9.2 ± 0.433	11.04 ± 0.406	11.2 ± 0.772	10.84 ± 0.553	12.28 ± 0.48	12.6 ± 0.707
Positive control 10 mg/kg	9.52 ± 0.205	11.32 ± 0.338	12.12 ± 1.23	12.16 ± 1.23	12.44 ± 0.934	13.08 ± 1.055

Table 4
Percentage inhibition for positive control, methanol: dichloromethane and aqueous extracts of the stinging nettle by comparing with the negative control group.

	Percentage inhibition at different time (hours)				
Extract	½ hour	1 h	1.5 h	2 h	2.5 h
Methanol: dichloromethane extract	16.81	0.766	5.73	2.66	−2.3
Aqueous extract	17.11	14.24	22.35	18.35	16.44
Positive control	15.01	7.19	12.89	17.28	13.26

and rhamnetin). The structures of the compounds are presented in Fig. 8.

In silico molecular docking

To identify, the specific bioactive compounds with anti-inflammatory potential, docking studies were conducted with indomethacin ligand used as the standard drug. The average binding energy for docking was -7.12 kcal/mol. Values that had the highest negative scores had a lower binding energy thus had a higher bioactivity. Quercetin, rhamnetin, quercetin rhamnoside, epigallocatechin gallate and chlorogenic acid had above average binding scores of -8.6, -8.5, -8.1, -8.0 and -8.3 kcal/mol respectively (Table 6). Therefore, they all had favorable binding energy. Protocatechuic had the lowest negative score of -3.8 kcal/mol and hence low biological activity.

Binding energy is the intermolecular force holding the receptor-ligand complex. The lower the binding energy, the higher the docking scores. The number of modes is the number of poses when the ligand orients to the binding site of the receptor during the flexible ligand fixed receptor docking.

Molecular interactions of quercetin and protocatechuic compared with indomethacin revealed the following: (i) Quercetin had similar interactions with indomethacin with hydrogen bonding at serine 530 (Table 7). Also, there was presence of distinct Pi-sulfur bond by interacting with methionine 522. (ii) Protocatechuic, it was devoid of hydrogen, Pi-Sulpur and Pi-Pi bonds (Figs. 9 and 10).

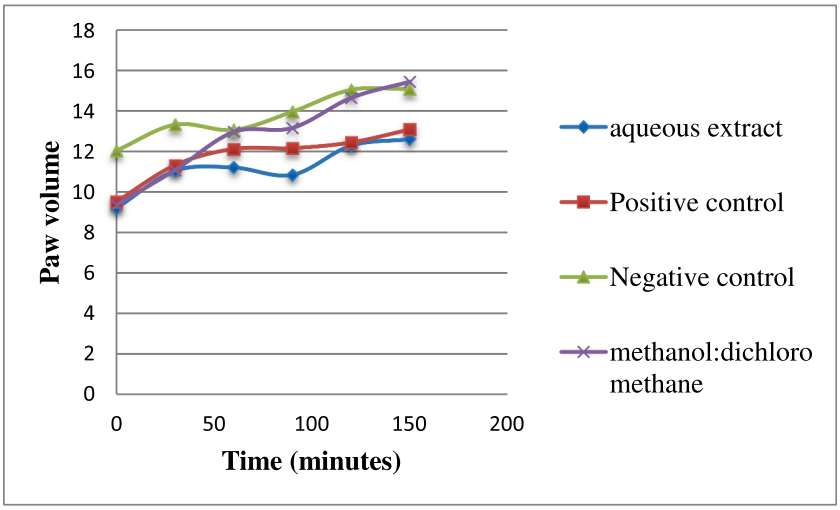

Fig. 6. Changes in paw volumes against time for aqueous and methanol: dichloromethane extracts of the stinging nettle, positive control, and negative control. All experiments were carried out using 5 replicates.

Table 5
The identified phytochemical compounds of the stinging nettle (*Urtica* species) extract using LC-MS analysis.

S/No.	Compound detected	Retention time (min)	Molecular formula	Molecular weight (g/mol)	Concentration (μg/g)	Mass to charge ratio (*M* + *H*)
1	Protocatechuic acid	0.35	$C_7H_6O_4$	154.12	0.0288	248.13
2	Gentisic acid	2.42	$C_7H_6O_4$	154.12	0.0212	308.08
3	Syringic acid	5.33	$C_9H_{10}O_5$	198.17	0.0198	342.17
4	Gallic acid	5.62	$C_7H_6O_5$	170.12	0.0213	342.17
4	Caffeic acid	6.24	$C_9H_8O_4$	180.16	0.0373	566.22
5	p-Coumaric acid	8.13	$C_9H_8O_3$	164.05	0.1681	354.17
6	Chlorogenic acid	9.95	$C_{16}H_{18}O_9$	354.31	0.1654	348.12
7	Cinnamic acid	11.55	$C_9H_8O_2$	148.16	0.0702	487.21
8	Ferulic acid	11.95	$C_{10}H_{10}O_4$	194.18	0.0567	611.20
9	Sinapic acid	12.16	$C_{11}H_{12}O_5$	224.21	0.0302	487.21
10	Isorhamnetin rutinoside	14.39	$C_{28}H_{32}O_{16}$	624.5	0.2134	467.19
11	Kaempferol-3-rutinoside	15.81	$C_{27}H_{30}O_{15}$	594.5	0.0352	409.16
12	Quercetin rhamnoside	18.04	$C_{21}H_{20}O_{11}$	448.4	1.4785	218.15
13	Kaempferol hexoside	20.10	$C_{21}H_{20}O_{11}$	448.4	1.1314	224.20
14	Myricetin	21.37	$C_{15}H_{10}O_8$	318.237	0.0412	350.14
15	Quercetin	21.64	$C_{15}H_{10}O_7$	302.236	0.0276	252.23
16	Quercetin-3 glucoside	25.30	$C_{21}H_{19}O_{12}$	463.4	0.0137	552.31
17	Rhamnetin	25.78	$C_{16}H_{12}O_7$	316.26	0.0167	413.27
18	Quercetin	27.44	$C_{15}H_{10}O_7$	302.236	0.0313	584.34
19	Epigallocatechin gallate	28.69	$C_{22}H_{18}O_{11}$	458.372	0.0297	248.13

Discussion

DNA barcoding is a novel approach for identifying and discriminating species based on the nucleotide diversity of conserved sequences. Several studies have indicated that DNA barcodes *rbcL* and ITS2 based on the chloroplast-plastid and nuclear regions, respectively, have been used to identify various plant families with similar morphological traits [42,43]. Based on *rbcL* and ITS sequences the stinging nettle sample used in this study was identified as *Urtica* species. This finding is consistent with a previous study that sampled stinging nettle from Kenya and performed molecular phylogenetic analysis based on DNA barcoding markers [20]. Other nettle species growing in Africa include *Urtica massaica Mildbr* in Kenya [44] and *Urtica simensis* in Ethiopia [45].

The phenolic content of methanol: dichloromethane extract of *Urtica* sp. was found to be within the range for methanol: dichloromethane extracts of *Urtica dioica* at 0.24 ± 0.15 - 100.30 ± 0.01 mg GAE/g extracts [46] and 2.18–4.84 mg GAE for *Urtica simensis* [47]. Therefore, the phenolic acids do not vary quantitatively at the species level for the *Urticaceae* family. On the other hand, flavonoid content of methanol: dichloromethane extract of *Urtica* sp. registered a higher concentration than the previously reported contents from methanolic extracts of *Urtica dioica* at 0.0081–0.0180 mg quercetin/g [48]. This discrepancy reflects on the

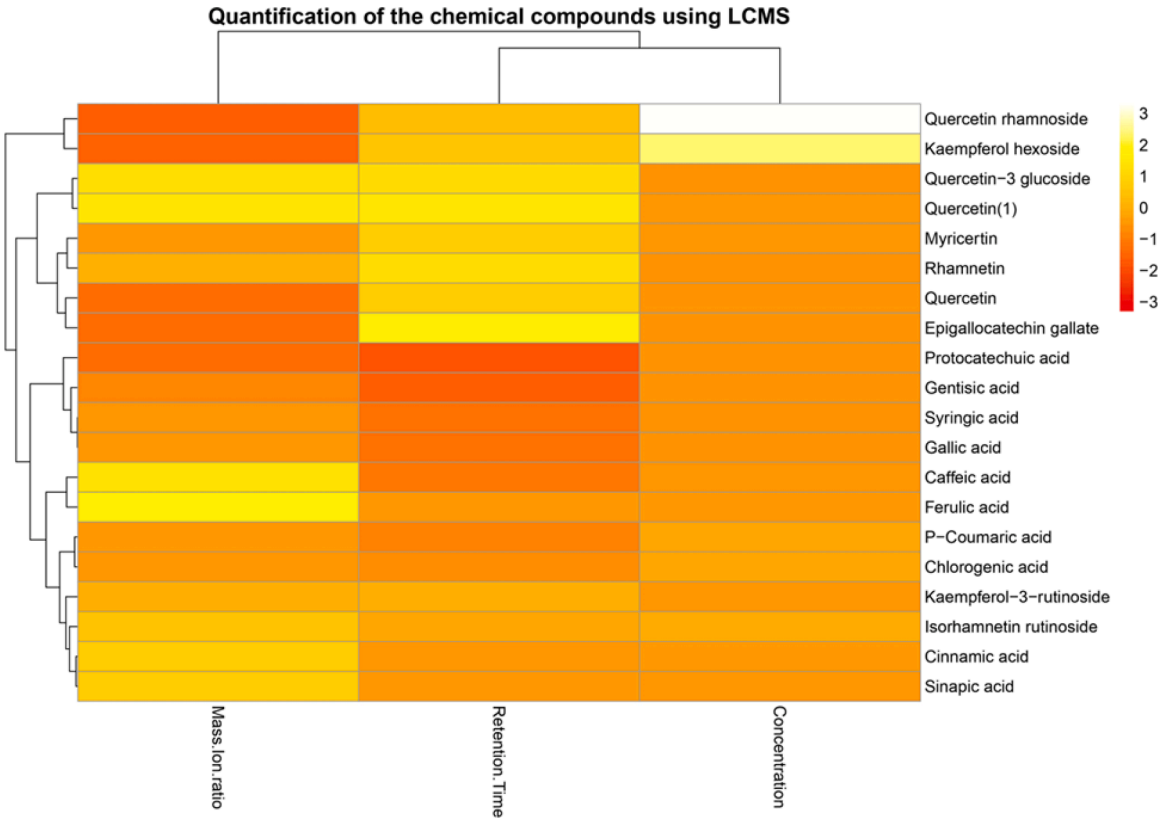

Fig. 7. Heat map representation for the identified compounds of *Urtica* sp. using LC-MS analysis.

phytochemical diversity of the Urticaceae family whereby polar flavonoids are highly concentrated and there are significant amount of phenolic acids in Kenyan *Urtica* species. In addition, previous reports demonstrated that the chemodiversity of *Urtica massaica* is not dependent on the geographical variations [49]. Polyphenols have been shown to be potent inhibitors of nitric oxide synthetase activity and nitric oxide production which have been shown to be implicated in tissue damage and inflammation [1]. Also flavonoids possess potent inhibitory activity against a wide array of enzymes and prostaglandins [1]. The phytochemical investigations of the methanol: dichloromethane extracts of *Urtica* sp. revealed important levels of polyphenols and flavonoids compared with previous reports [47, 48].

The current study demonstrated the anti-inflammatory activity of *Urtica* species aqueous extract against carrageenan-caused paw edema. The edema formation in the rat paw is a biphasic event, firstly mediated by the release of serotonin and histamine and secondly, it is attributed to the release of prostaglandins implicated in the inflammatory procedure, which is directly related to inhibiting the production of prostaglandin synthesis induced by COX-1 and COX-2 [50]. The high percentage inhibition rate of the aqueous extract implies that it is the most potent extract in anti-inflammatory activity. The *Urtica* sp. aqueous extract inhibition of inflammation was found to be greater than that induced by the reference drug. Therefore, this could be partly caused by the decrease of the release of inflammatory mediators by phytochemical compounds in *Urtica* sp. extract. Similarly, it was reported that the water-soluble extract of *Urtica dioica* suppressed the NF-κB activated by tumor necrosis factor (TNF) and lipopolysaccharide (LPS) [51]. Another study suggested that the lipophilic phase is not only more potent but also non-cytotoxic [52]. The *in vivo* carrageenan model involves the activation of cyclooxygenases and lipoxygenases. Its development occurs in a biphasic fashion. The first phase lasts within the first 50 min due to the action of neurotransmitters, histamine and serotonin. The remaining phase is as a result of the prostaglandins and bradykinins activity [50]. From the results obtained in this study, the anti-inflammatory activity of the extract may be due to the inhibition of prostaglandins and kinins since it started from the second hour. The aqueous extract appeared to have a long duration of anti-inflammatory action evidenced by the fact that the anti-inflammatory activity of all test doses increased with time. The Carrageenan model can be used to indicate not only the degree but also the duration of anti-inflammatory action [31].

The analysis of phytochemical composition of the aqueous extracts by LC-MS confirmed the presence of phenolic and flavonoid compounds and their derivatives with a total of 19 compounds identified. Among them, the major peaks area in the chromatogram were chlorogenic acid, quercetin-3-O rhamnosyl glucoside / rutin and isoquercetin, rutin and chlorogenic acid. These major compounds identified in this study have also been reported in *Urtica dioica* extract [53–55]. In contrast to *Urtica* sp., the most abundant compounds in *Urtica dioica* are 2-O caffeoyl malic acid, chlorogenic acid, and rutin [56]. Quercetin and rutin have beneficial effects in different cell cultures and animal models of inflammation [57,58]. These data were correlated to an earlier study in which *Urtica dioca*

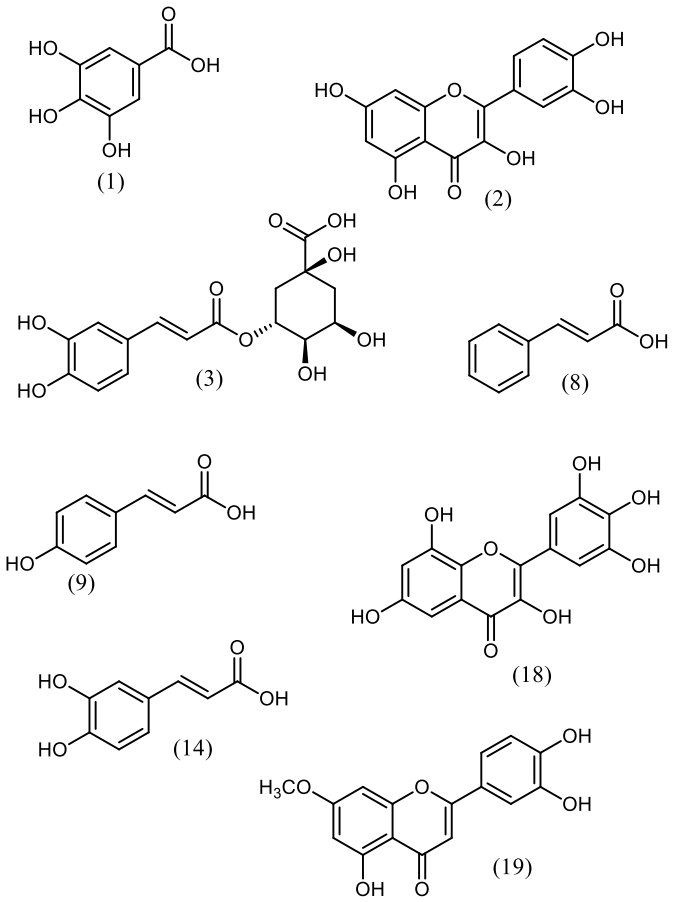

Fig. 8. Chemical structures of selected compounds (1 = gallic acid, 2 = quercetin, 3 = 3-caffeoylquinic acid, 8 = cinnamic acid, 9 = *p*-coumaric acid, 14 = chlorogenic acid, 18 = myricetin, 19 = rhamnetin) from the stinging nettle identified by Raman Spectroscopy and LC MS.

Table 6
Molecular docking binding energy and number of modes results of the stinging nettle polyphenols and indomethacin.

Ligand	Binding energy (kcal/mol)	Number of modes
Protocatechuic acid	-3.8	9
Gentisic acid/dihydroxybenzoic acid	-6.3	9
Syringic acid	-6.1	9
Gallic acid	-6.6	9
Caffeic acid	-7.2	9
P-Coumaric acid/ 4 hydroxycinnamic acid	-7.0	9
Chlorogenic acid	-8.3	9
Cinnamic acid	-6.8	9
Ferulic acid	-7.1	9
Sinapic acid	-6.7	9
Isorhamnetin rutinoside (Isorhamnetin-3-O-rutinoside)	-6.9	2
Kaempferol-3-rutinoside	-7.4	3
Quercetin rhamnoside (Quercetin 3- rhamnoside)	-8.1	7
Kaempferol hexoside (kaempferol 3-O hexoxyl hexoside)	-6.9	6
Myricetin	-7.9	9
Quercetin	-8.6	9
Quercetin-3 glucoside	-7.1	8
Rhamnetin	-8.5	9
Epigallocatechin gallate	-8.0	9

Table 7
Interaction types and amino acids involved in the inhibition of COX-2 by quercetin, indomethacin and protocatechuic.

Interaction type	Van der Waal forces	Hydrogen	Pi-sulfur	Pi-Pi	Pi –sigma	Pi-alkyl	CH
Quercetin	Tyrosine 348 Valine 349 Tyrosine 355 Histidine 90 Arginine 513 Glycine 192 Alanine 516 Isoleucine 517 Phenylalanine 518 Leucine 384 Glycine 526 Phenylalanine 381	Serine 530 Tyrosine 385	Methionine 522	Valine 523 Leucine 352	Tryptophan 387	Valine 523	–
Indomethacin	Valine 116 Tyrosine 355 Leucine 352 Tyrosine 348 Glycine 520 Arginine 120 Serine 353 Phenylalanine 518 Leucine 359	Serine 530	–	Tyrosine 385 Tryptophan 387	Valine 349 Alanine 527	Phenyl 381 Methionine 522 Leucine 384, 352 Valine 523	Phenylalanine 518 Tyrosine 348
Protocatechuic	Asparagine 375 Phenylalanine 529 Glycine 533 Serine 530 Glycine 227	–	–	–	Phenylalanine 209	Phenylalnine 381 Leucine 534 Valine 228 Isoleucine 337 Phenylalanine 209	–

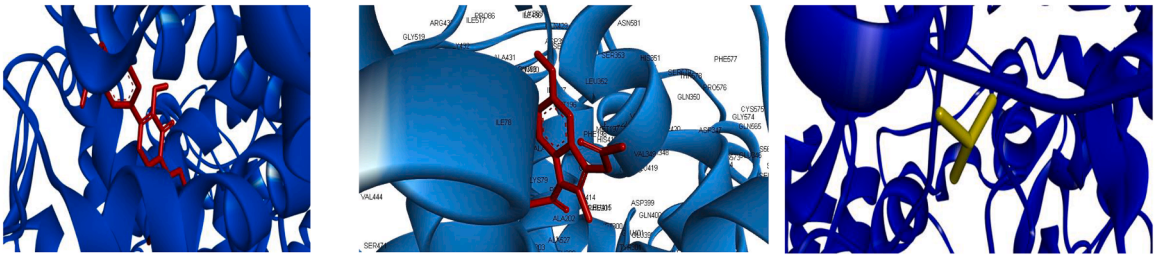

Fig. 9. Three dimensional (3D) representation of cyclooxygenase docked with quercetin (left), indomethacin (middle) and protocatechuic (right) using BIOVIA Discovery Studio 2021 Client.

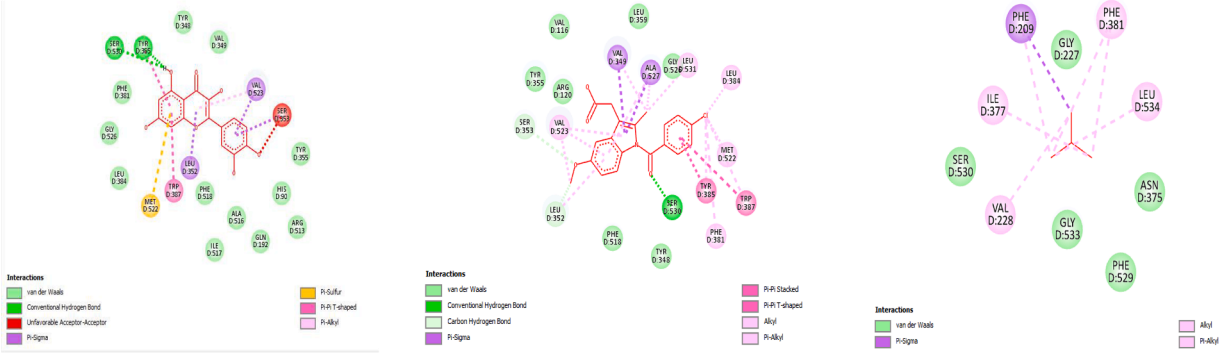

Fig. 10. Two dimensional (2D) representation of cyclooxygenase docked with quercetin (left), indomethacin (middle) and protocatechuic (right) using BIOVIA Discovery Studio 2021 Client.

was rich in phenolic compounds such as quercetin and several studies have indicated that these polyphenols are mainly accountable for the anti-inflammatory properties of many medicinal plants [57,58]. Polyphenols can act as anti-inflammatory agent by inhibiting the secretion of pro-inflammatory mediators (cytokines, prostaglandins), regulating the activities of inflammation related cells, chelating free radical mediators and inhibiting the activity of inflammatory enzymes [59]. These effects confirm that polyphenols play a significant role in prevention and control of inflammation. Hydromethanolic extracts of *Urtica massaica* revealed the presence of anthocyanins, flavonoids, saponosides and tannins [60]. Previous reports from *in vivo* animal studies demonstrated that quercetin reduces inflammation caused by carrageenan and high fat diet by inhibiting production of cyclooxygenases and lipoxygenases [61]. In another study, it was found that the *Urtica dioica* extract had greater anti-inflammatory activity than quercetin. This suggests that the anti-inflammatory activity is as a result of synergism amongst several compounds instead of a single compound [62]. A commercial preparation of the nettle leaf (IDS23) was compared against caffeoyl-malic isolated from the leaf for their antiphlogistic effects. It was found that the IDS23 partially inhibited 5-lipoxygenase through several bioactive phytochemicals including caffeoyl-malic acid [63]. Stewed *Urtica dioica* was also found to enhance the anti-rheumatic activity of diclofenac, an NSAID, in treating acute arthritis [64]. Thus, all compounds identified in the analysis of *Urtica* sp. through LC-MS have already been described in the reviewed literature of different extracts of the *Urtica* species, which corroborates the findings of anti-inflammatory in other studies.

Molecular docking has the potential as a screening tool for prediction of the effect of pharmacological candidate on cells. The results of the predictions are important in the design of experiments to evaluate the likelihood of false positives in the selected chemical leads for biological activity. The binding affinity of the bioactive compounds to their respective protein targets determines the scores of the compound [65]. In the current study, the most potent bioactive anti-inflammatory compounds were quercetin, rhamnetin, quercetin rhamnoside, epigallocatechin gallate and chlorogenic acid since they exhibited low binding energy when they were docked into COX-2 proteins to form the complexes *in silico*. Our findings demonstrated that these compounds bind steadily to the target gene's active pockets highlighting the possibility that these substances could be used to treat inflammation by inhibiting COX-2 gene. From the perspective of this study, it has been revealed that Quercetin possesses higher docking score due to its lowest binding energy with the target COX-2. Overall, it can be stated from the analogy of both scores that Quercetin can be a highly potent agent against inflammation. Additionally, the presence of hydrogen and Pi-sulfur bonds with different amino acid residues stabilize the docking molecular interactions. These observations demonstrate that Quercetin is a potential drug candidate as an anti-inflammatory agent by inhibiting cyclooxygenase. Quercetin rhamnoside has been reported as an anti-oxidant and anti-inflammatory compound [66]. Apart from cyclooxygenase, quercetin modulates the expression of p65-Nuclear Factor NF-kappa-B thus having anti-inflammatory properties [67]. Similarly, epigallocatechin attenuates inflammation by reducing activation of NF-κB pathway in mesangial cells of lupus like mice [68]. Therefore, the selected compounds from *Urtica* species in this study have excellent docking scores and could be potent candidates for further studies to evaluate as natural anti-inflammation drugs for humans.

The limitations of the current study are: The levels of bioactive compounds in *Urtica* species have been documented to be affected by extraction solvents and method of extraction. Many different solvents (e.g., methanol, ethanol, water) and some methods (Soxhlet extraction, microwave) have been investigated to improve the recovery of bioactive compounds in *Urtica* species. Future efforts should focus on the identification of optimal conditions (e.g., extraction methods, solvents) to maximize the recovery of bioactive compounds in *Urtica* species. Further studies are important in relation to: (i) formulations of effective dosage forms; and (iii) safety of *Urtica* species extracts.

Conclusion and future directions

The present study demonstrated that *Urtica* sp. found in Kenya is rich in a variety of phenolic and flavonoid compounds, the aqueous crude extract has high anti-inflammatory properties than methanol dichloromethane extract, and quercetin attained the best docking score with COX-2 and hence the most potent anti-inflammatory compound. The anti-inflammatory properties of the extracts suggest that *Urtica* species and its by-products could be an excellent source of raw materials for the nutraceuticals and pharmaceutical industries. Taking together these results, the novelty of this research relies on demonstrating, for the first time, the therapeutic application of Kenyan *Urtica* species using *in vivo* and *in silico* models. The findings of this work can have several applications in pharmacognosy and pharmacotherapy.

Identification of the *Urtica* plant species in this study relied on DNA barcoding based on *rbcL* and ITS2 barcode regions. According to the analysis, *rbcL* and ITS2 sequences cannot completely identify the *Urtica* species. The genus of the plant sample was identified as *Urtica*, with no discrimination at the species level. We have provided this information in the manuscript and provided the following recommendation for future research. Therefore, there is need to use a multi-approach based on morphological and other DNA barcoding markers including nuclear and chloroplast regions such as *matK* and *trnH-psbA* to improve on the resolution for species identification.

The *in vivo* and *in silico* studies showed that the aqueous extract of Urtica species possesses a greater anti-inflammatory effect that the reference drug (indomethacin). Therefore these results could be exploited in the development of new natural pharmaceutical drugs that have anti-inflammatory activities with fewer side effects. Thus there is need to isolate individual bioactive compounds, test their anti-inflammatory activities and mechanisms of action. In order to further characterize the anti-inflammatory properties of *Urtica* species, future research will focus on the identification and purification of compounds driving the inhibition of cytokine secretion. Furthermore, studies on synergistic phytochemical interactions can provide clues in elucidating the anti-inflammatory action of *Urtica* species.

The phytochemical profile of *Urtica* species analyzed via LC-MS had a high degree of similarities to the already identified compounds, so that there was no significant difference between the phytochemical profiles of the species studied and the reports in the

literature. Further study and new tests are required to evaluate the anti-inflammatory activity with respect to different parts of the plant and fractions or groups of compounds present in the *Urtica* species.

Urtica species aqueous extract exhibited significant *in vivo* anti-inflammatory action and this action was confirmed by molecular docking studies, in which quercetin and protocatechuic, identified in *Urtica* species by LC-MS, showed good profiles for COX-2 enzyme inhibition. However, with regard to the experiments, other mechanisms of inflammatory induction should be tested at different doses and using different fractions or compounds of the extract of the species studies for a better understanding of the profile and potential of secondary metabolites present in the species. There is also need to utilize different *in vivo* acute and chronic models of inflammation to explore a variety of anti-inflammatory mechanisms.

CRediT authorship contribution statement

Jacqueline Wambui: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Robert I.O. Ikedi:** Methodology, Validation, Writing – review & editing. **Rosaline W. Macharia:** Conceptualization, Methodology, Investigation, Validation, Formal analysis, Supervision, Writing – review & editing. **Francisca Kama-Kama:** Conceptualization, Methodology, Investigation, Validation, Formal analysis, Supervision, Writing – review & editing. **Evans N. Nyaboga:** Conceptualization, Methodology, Investigation, Validation, Formal analysis, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to acknowledge the Faculty of Science and Technology (Department of Biochemistry and Department of Physics) and Faculty of Health Sciences (Department of Pharmacy) at the University of Nairobi for laboratory space and equipment for the study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2024.e02088.

References

[1] J.H. Jang, T.J. Lee, Mechanisms of phytochemicals in anti-inflammatory and anti-cancer, Int. J. Mol. Sci. 24 (9) (2023), https://doi.org/10.3390/ijms24097863.
[2] J.C. Maroon, J.W. Bost, A. Maroon, Natural anti-inflammatory agents for pain relief, Surg. Neurol. Int. 1 (2010), https://doi.org/10.4103/2152-7806.73804.
[3] G. Yuan, M.L. Wahlqvist, G. He, M. Yang, D. Li, Natural products and anti-inflammatory activity, Asia Pac. J. Clin. Nutr., 15 (2) (2006) 143–152.
[4] R. Pahwa, A. Goyal, I. Jialal, Chronic inflammation. StatPearls, StatPearls Publishing Treasure Island, 2022.
[5] A. Attiq, J. Jalil, K. Husain, W. Ahmad, Raging the war against inflammation with natural products, Front. Pharmacol. 9 (2018), https://doi.org/10.3389/fphar.2018.00976.
[6] I. Dona, M. Salas, J.R. Perkins, E. Barrionuevvvo, F. Gaeta, J.A. Cornejo-Garcia, P. Campo, M.J. Torres, Hypersensitivity reactions to non steriodal anti inflammatory drugs, Curr. Pharm. Des. 22 (45) (2016) 6784–6802, https://doi.org/10.2174/1381612822666160928142814.
[7] A.L. Buchman, Side effects of corticosteroids therapy, J. Clin. Gastroenterol. 33 (4) (2023) 289–294, https://doi.org/10.1097/00004836-200110000-00006.
[8] M.G. Hillinger, R.Q. Wolever, L.C. McKernan, R. Elam, Integrative medicine for the treatment of persistent pain, Prim. Care 44 (2) (2017) 247–264, https://doi.org/10.1016/j.pop.2017.02.008.
[9] C. Di Lorenzo, M. Dell'Agli, M. Badea, L. Dima, E. Colombo, E. Sangiovanni, P. Restani, E. Bosisio, Plant food supplements with anti-inflammatory properties: a systematic review (II), Crit. Rev. Food Sci. Nutr. 53 (5) (2013) 507–516, https://doi.org/10.1080/10408398.2012.691916.
[10] K. Rayburn, E. Fleischbein, J. Song, B. Allen, M. Kundert, C. Leiter, T. Bush, Stinging nettle cream for osteoarthritis, Altern. Ther. Health Med. 15 (4) (2009). PMID: 19623834.
[11] A. Jacquet, P.O. Girodet, A. Pariente, K. Forest, L. Mallet, N. Moore, Phytalgic®, a food supplement, *vs* placebo in patients with osteoarthritis of the knee or hip: a randomised double-blind placebo-controlled clinical trial, Arthritis Res. Ther. 11 (6) (2009), https://doi.org/10.1186/ar2891.
[12] S.A. Dar, F.A. Ganai, A.R. Yousuf, M.U.H. Balkhi, T.M. Bhat, P. Sharma, Pharmacological and toxicological evaluation of *Urtica dioica*, Pharm. Biol. 51 (2) (2013) 170–180, https://doi.org/10.3109/13880209.2012.715172.
[13] E.O. Omwenga, A. Hensel, A. Shitandi, F.M. Goycoolea, Ethnobotanical survey of traditionally used medicinal plants for infections of skin, gastrointestinal tract, urinary tract and the oral cavity in Borabu sub-county, Nyamira county, Kenya, J. Ethnopharmacol. 176 (2015) 508–514, https://doi.org/10.1016/j.jep.2015.11.032.
[14] G.J.H. Grubben, Plant resources of tropical Africa 2 vegetables. PROTA (2004), pp. (540).
[15] K.R. Patil, U.B. Mahajan, B.S. Unger, S.N. Goyal, S. Belemkar, S.J. Surana, S. Ojha, C.R. Patil, Animal models of inflammation for screening of anti-inflammatory drugs: implications for the discovery and development of phytopharmaceuticals, Int. J. Mol. Sci. 20 (18) (2019), https://doi.org/10.3390/ijms20184367.
[16] A. Panthong, P. Norkaew, D. Kanjanapothi, T. Taesotikul, N. Anantachoke, V. Reutrakul, Anti-inflammatory, analgesic and antipyretic activities of the extract of gamboge from *Garcinia hanburyi* Hook f, J. Ethnopharmacol. 111 (2) (2007) 335–340, https://doi.org/10.1016/j.jep.2006.11.038.
[17] R. Boominathan, B. Parimaladevi, S.C. Mandal, S.K. Ghoshal, Anti-inflammatory evaluation of *Ionidium suffruticosam* Ging. in rats, J. Ethnopharmacol. 91 (2–3) (2004) 367–370, https://doi.org/10.1016/j.jep.2003.12.019.
[18] S. Sarkhel, Evaluation of the anti-inflammatory activities of *Quillaja saponaria* Mol. saponin extract in mice, Toxicol. Rep. 3 (1–3) (2016), https://doi.org/10.1016/j.toxrep.2015.11.006.

[19] F. Kama-Kama, J. Midiwo, J. Nganga, N. Maina, E. Schiek, L.K. Omosa, G. Osanjo, J. Naessens, Selected ethno-medicinal plants from kenya with *in vitro* activity against major African livestock pathogens belonging to the "*Mycoplasma mycoides* Cluster, J. Ethnopharmacol. 192 (2016) 524–534, https://doi.org/10.1016/j.jep.2016.09.034.

[20] Z.Y. Wu, A.K. Monro, R.I. Milne, H. Wang, T.S. Yi, J. Liu, D.Z. Li, Molecular phylogeny of the nettle family (Urticaceae) inferred from multiple loci of three genomes and extensive generic sampling, Mol. Phylogenet. Evol. 69 (3) (2013) 814–824, https://doi.org/10.1016/j.ympev.2013.06.022.

[21] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT, Nucleic Acids Symp. Ser. 41 (41) (1999) 95–98.

[22] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, Basic local alignment tool, J. Mol. Biol. 215 (3) (1990) 403–410, https://doi.org/10.1016/S00222836(05)80360-2.

[23] F. Kama-Kama, L.K. Omosa, J. Nganga, N. Maina, G. Osanjo, S. Yaouba, M. Ilias, J. Midiwo, J. Naessens, Animycoplasmal activities of compounds from *Solanum aculeastrum* and *Piliostigma thonningii* against Strains from the *Mycoplasma mycoides* Cluster, Front. Pharmacol. 8 (2017).

[24] M.A. Ebrahimzadeh, M. Gharekhani, M. Ghorbani, P. Dargany, Effect of extract of aerial parts of *Urtica dioica* (Urticaceae) on the stability of soyabean oil, Trop. J. Pharm, Res. 14 (1) (2015) 125–131, https://doi.org/10.4314/tjpr.v14i1.18.

[25] S. Telo, I. Halifeoglu, I.H. Ozercan, Effects of stinging Nettle (*Urtica dioica* L.,) on antioxidant enzyme activities in rat model of mammary gland cancer, Iran. J. Pharm. Res. 16 (2017) 164–170.

[26] S. Sangeeta, V. Vrunda, Quantitative and qualitative analysis of phenolic and flavonoid content in *Moringa oleifera* Lam and *Ocimum tenuiflorum* L, Pharmacogn. Res. 8 (1) (2016) 16–21, https://doi.org/10.4103/0974-8490.171095.

[27] B.A. Hanson, ChemoSpec: Exploratory Chemometrics for Spectroscopy. R Package Version 4.1.15, 2015.

[28] OriginPro, Version, OriginLab Corporation, Northampton, MA, USA, 2023.

[29] O.O. Adeyemi, S.O. Okpo, O.O. Ogunti, Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae), Fitoterapia 73 (5) (2002) 375–380, https://doi.org/10.1016/S0367-326X(02)00118-1.

[30] S. Amdekar, P. Roy, V. Singh, A. Kumar, R. Singh, P. Sharma, Anti-inflammatory activity of *Lactobacillus* on carrageenan-induced paw edema in male Wistar rats, Int. J. Inflam. 2012 (2012), https://doi.org/10.1155/2012/752015.

[31] C.A. Winter, E.A. Risley, G.W. Nuss, Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547, https://doi.org/10.3181/00379727-111-27849.

[32] G.R.M. Pérez, Anti-Inflammatory Activity of *Ambrosia artemisiaefolia* and *Rhoeo spathacea*, Phytomed. Int. J. Phytother. Phytopharm. 3 (2) (1996) 163–167, https://doi.org/10.1016/S0944-7113(96)80030-4.

[33] Agilent Technologies. Sample Preparation Fundamentals for Chromatography (2013).

[34] J. Siji, Agilent 6120B single quadrupole walkup system for multi-user labs. Agilent Technologies 5991-6535EN.

[35] F. Nantel, D. Denis, R. Gordon, A. Northey, M. Cirino, K.M. Metters, C.C. Chan, Distribution and regulation of cyclooxygenase-2 in carrageenan-induced inflammation, Br. J. Pharmacol. 128 (1999) 853–859, https://doi.org/10.1038/sj.bjp.0702866.

[36] R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson, W. C. Stallings, Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents, Nature 384 (6610) (1996) 644–648, https://doi.org/10.1038/384644a0.

[37] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Godsell, A.J. Olson, AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility, J. Comput. Chem. 30 (16) (2009) 2785–2791, https://doi.org/10.1002/jcc.21256.

[38] O. Trott, A.J. Olson, AutoDock vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, J. Comput. Chem. 31 (2) (2010) 455–461, https://doi.org/10.1002/jcc.21334.

[39] B. Diallo, T. Swart, H. Hoppe, O.T. Bishop, K. Lobb, Potential repurposing of four FDA approved compounds with antiplasmodial activity identified through proteome scale computational drug discovery and *in vitro* assay, Sci. Rep. 11 (1) (2021) 655, https://doi.org/10.1038/s41598-020-80722-2.

[40] P.J. Eravuchira, R.M. El-Abassy, S. Deshpande, M.F. Matei, S. Mishra, P. Tandon, N. Kuhnert, A. Materny, Raman spectroscopic characterization of different regioisomers of monoacyl and diacyl chlorogenic acid, Vib. Spectrosc. 61 (2012) 10–16, https://doi.org/10.1016/j.vibspec.2012.02.009.

[41] D. Pompeu, Y. Larondelle, H. Rogez, O. Abbas, J.A. Fernández Pierna, V. Baeten, Characterization and discrimination of phenolic compounds using fourier transform raman spectroscopy and chemometric tools, Biotechnol. Agron. Soc. Environ. 22 (1) (2018) 13–28.

[42] H. Yao, J. Song, Use of ITS2 region as the universal DNA barcode for plants and animals, PLoS One 5 (2010) e13102.

[43] K.W. Nderitu, E. Ager, E. Mecha, A. Nyachieo, DNA barcoding using ITS2 and RBCL markers for Solanaceae species identification, East Afr. Med. J. 100 (1) (2023).

[44] G. Njoroge, Traditional medicinal plants in two urban areas in Kenya (Thika and Nairobi): diversity of traded species and conservation concerns, Ethnobot. Res. Appl. 10 (2012) 329–338.

[45] T. Keflie, Stinging nettle (*Urtica Simensis*) as potential resource of vitamin E, Curr. Dev. Nutr. 4 (2020), https://doi.org/10.1093/cdn/nzaa067_042.

[46] A.M. Chahardehi, D. Ibrahim, S.F. Sulaiman, Antioxidant activity and total phenolic content of some medicinal plants in urticaceae family, J. Appl. Biol. Sci. 3 (2) (2009) 27–31.

[47] S. Fattahi, E. Zabihi, Z. Abedian, R. Pourbagher, A.A. Motevalizadeh, A. Mostafazadeh, H. Akhavan-Niaki, Total phenolic and flavonoid contents of aqueous extract of stinging nettle and *in vitro* antiproliferative effect on hela and BT-474 cell lines, Int. J. Mol. Cell. Med. 3 (2) (2014) 102–107.

[48] S. Begić, E. Horozić, H. Alibašić, E. Bjelić, S. Seferović, E.C. Kozarević, M. Ibišević, A. Zukić, E. Karić, M. Softić, Antioxidant capacity and total phenolic and flavonoid contents of methanolic extracts of *Urtica dioica* L. by different extraction techniques, Int. Res. J. Pure Appl. Chem. 21 (23) (2020) 207–214, https://doi.org/10.9734/irjpac/2020/v21i2330319.

[49] A. Kipruto, The effect of soil and climatic factors on antimicrobial and phytochemical composition of stinging nettle (*Urtica massaica*). Thesis, University of Eldoret (2019).

[50] A. Gepdiremen, V. Mshvildadze, H. Süleyman, R. Elias, Acute and chronic antiinflammatory effects of *Hedera colchica* in rats, J. Ethnopharmacol. 94 (1) (2004) 191–195, https://doi.org/10.1016/j.jep.2004.06.001.

[51] K. Riehemann, B. Behnke, K. Schulze-Osthoff, Plant extracts from stinging nettle (*Urtica dioica*), an antirheumatic remedy, inhibit the pro-inflammatory transcription factor NF-Kappa β, FEBS Lett. 442 (1) (1999) 89–94, https://doi.org/10.1016/s0014-5793(98)01622-6.

[52] T.A. Johnson, J. Sohn, W.D. Inman, L.F. Bjeldanes, K. Rayburn, Lipophilic stinging nettle extracts possess potent anti-inflammatory activity, are not cytotoxic and may be superior to traditional tinctures for treating inflammatory disorders, Phytomed. Int. J. Phytother. Phytopharm. 20 (2) (2013) 143–147, https://doi.org/10.1016/j.phymed.2012.09.016.

[53] M. Francišković, R. Gonzalez-Pérez, D. Orčić, F. Sánchez de Medina, O. Martínez- Augustin, E. Svirčev, N. Simin, N. Mimica-Dukić, Chemical composition and immuno-modulatory effects of *Urtica dioica* L. (stinging nettle) extracts, Phytother. Res. 31 (8) (2017) 1183–1191, https://doi.org/10.1002/ptr.5836.

[54] D. Orčić, M. Francišković, K. Bekvalac, E. Svirčev, I. Beara, M. Lesjak, N. Mimica-Dukić, Quantitative determination of plant phenolics in *Urtica dioica* extracts by high-performance liquid chromatography coupled with tandem mass spectrometric detection, Food Chem. 143 (2017) 48–53, https://doi.org/10.1016/j.foodchem.2013.07.097.

[55] S. Otles, B. Yalcin, Phenolic compounds analysis of root, stalk, and leaves of nettle, Sci. World J. (2012) e564367, https://doi.org/10.1100/2012/564367.

[56] U.J. Vajić, J. Grujić-Milanović, J. Živković, K. Šavikin, D. Gođevac, Z. Miloradović, B. Bugarski, N. Mihailović-Stanojević, Optimization of extraction of stinging nettle leaf phenolic compounds using response surface methodology, Ind. Crops Prod. 74 (2015) 912–917, https://doi.org/10.1016/j.indcrop.2015.06.032.

[57] T. Chen, X. Zhang, G. Zhu, H. Liu, J. Chen, Y. Wang, X. He, Quercetin inhibits TNF-α induced HUVECs apoptosis and inflammation via downregulating NF-kB and AP-1 signaling pathway *in vitro*, Medicine 99 (38) (2020), https://doi.org/10.1097/MD.0000000000022241 (Baltimore).

[58] N.O. Al-Harbi, F. Imam, M.M. Al-Harbi, O.A. Al-Shabanah, M.R. Alotaibi, H.M. As Sobeai, M. Afzal, I. Kazmi, A.C. Al Rikabi, Rutin inhibits carfilzomib-induced oxidative stress and inflammation via the NOS-mediated NF-κB signaling pathway, Inflammopharmacology 27 (4) (2019) 817–827, https://doi.org/10.1007/S10787-018-0550-5.

[59] J. Wang, J. Xu, X. Gong, M. Yang, C. Zhang, M. Li, J. Wang, J. Xu, X. Gong, M. Yang, C. Zhang, Biosynthesis, chemistry, and pharmacology of polyphenols from chinese salvia species: a review, Molecules 24 (1) (2019), https://doi.org/10.3390/molecules24010155.
[60] A. Nahayo, M.J. Bigendako, K. Fawcett, H. Nkusi, J.B. Nkurikiyimfura, G. Yansheng, Chemical study of the stems of *Urtica massaica*, a medicinal plant eaten by mountain gorillas (Gorilla Beringei Beringei) in Parc National Des Volcans, Rwanda. Res. J. Appl. Sci. 3 (7) (2008) 514–520.
[61] Y. Li, J. Yao, C. Han, J. Yang, M.T. Chaudhry, S. Wang, H. Liu, Y. Yin, Quercetin, Inflammation and Immunity, Nutrients 8 (3) (2016), https://doi.org/10.3390/nu8030167.
[62] L.G. Cuinica, R.B. Chissico, Anti-inflammatory properties of extract and quercetin from *Urtica dioica* L, Adv. Anal. Pharm. Chem. (2018).
[63] G.M. Kavalali, Urtica: The Genus Urtica, CRC Press, 2003.
[64] S. Chrubasik, W. Enderlein, R. Bauer, W. Grabner, Evidence for antirheumatic effectiveness of Herba *Urticae dioicae* in acute arthritis: a pilot study, Phytomedicine 4 (2) (1997) 105–108, https://doi.org/10.1016/S0944-7113(97)80052-9.
[65] D. Salaria, R. Rolta, C.N. Patel, K. Dev, A. Sourirajan, V. Kumar, *In vitro* and *in silico* analysis of *Thymus serpyllum* essential oil as bioactivity enhancer of antibacterial and antifungal agents, J. Biomol. Struct. Dyn. 40 (2021) 10383–10402.
[66] G.E.S. Batiha, A.M. Beshbishy, M. Ikram, Z.S. Mulla, M.E.A. El-Hack, A.E. Taha, A.M. Algammal, Y.H.A. Elewa, The pharmacological activity, biochemical properties, and pharmacokinetics of the major natural polyphenolic flavonoid: quercetin, Foods 9 (3) (2020), https://doi.org/10.3390/foods9030374.
[67] A. Bastin, M. Teimouri, S. Faramarz, M. Shabani, A.H. Doustimotlagh, A. Sadeghi, *In vitro* and molecular docking analysis of quercetin as an anti-inflammatory and antioxidant, Curr. Pharm. Des. 29 (11) (2023) 883–891, https://doi.org/10.2174/1381612829666230330084043.
[68] A. Peairs, R. Dai, L. Gan, S. Shimp, M.N. Rylander, L. Li, C.M. Reilly, Epigallocatechin-3- gallate (EGCG) attenuates inflammation in MRL/LPR mouse mesangial cells, Cell Mol. Immunol. 7 (2) (2010), https://doi.org/10.1038/cmi.2010.1.