

Antibacterial, antioxidant potency and chemical composition of essential oils from dried powdered leaves and flowers of *Hypericum revolutum* subsp. *keniense* (Schweinf.)

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Abstract

Hypericum revolutum subsp. *keniense* is a plant mainly used to treat diarrhoea, rheumatism, nervous disorders and wounds in African traditional medicine. The objective of the current work was to establish antibacterial, antioxidant potency and chemical composition of essential oil from the leaves and flowers of *Hypericum revolutum* subsp. *keniense*. The oils were isolated by steam distillation. Antibacterial activity against methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus* (ATCC 12393), *Escherichia coli* (ATCC 25922), *Acinetobacter baumannii* (ATCC 19606), *Salmonella* Enteritidis (NCTC12023), *Salmonella* Typhimurium (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 15442), and *Haemophilus influenzae* (ATCC 49766) were done by Agar Disk Diffusion and Microtiter Broth Dilution methods. Antioxidant activities of the essential oils were examined by different methods, DPPH, FRAP and H₂O₂ assays. Chemical characterization was done using Gas Chromatography interfaced with Mass Spectrometry, Fourier-Transform Infra-red (FTIR) spectroscopy and the quantification of phenolics and flavonoids by Folin-Ciocalteu reagent and Aluminium nitrate respectively. The oils showed potential antibacterial activity with mean zone of inhibition ranging from 20.67 ± 0.33 to 32.00 ± 1.00 mm at 100 % oil concentration against the tested bacteria. Furthermore, the minimum inhibitory concentrations (MICs) in all the tested microorganisms were in the range of 250 to 15.6250 µg/ml. The essential oils derived from the leaves revealed varied antioxidant activity levels with the different methods of assay. The IC₅₀ of values obtained from the three methods, DPPH, FRAP and H₂O₂ were 1000 µg/ml, 0.31 µg/ml and 12.33 µg/ml respectively. Caryophyllene (22.1 %) and 2,3,4-trimethylhexane were the major chemical components of the essential oils derived from the leaves and flowers respectively. FTIR spectroscopy of the essential oils from the leaves and flowers showed similarity at peaks for hydroxyl, unsaturated olefinic and amine functional groups. Further findings indicated that the total phenolic and flavonoid contents essential oils derived from leaves were 130.46 ± 10.5 mg GAE/g dry weight and 0.911 ± 0.04 mg CE/g dry weight respectively. It was therefore concluded that essential oils from the leaves and flowers of *H. revolutum* subsp. *keniense* have compounds that have antibacterial and antioxidant potency.

1.0 Introduction

Essential oils are mainly used as antimicrobial agents [1]. Also, they are used as insecticides, insect repellents, antimalaria, antidepressant, anticancer, antimutagenic, anti-inflammatory, analgesic, antipyretics, antioxidant, anticonvulsants, conjunctivitis, tuberculosis, stress, bronchitis, anxiety, neurological disorders, ulcers, liver conditions and Parkinson's disease [2–4]. The diverse biological activities of essential oils are attributed to the complex nature of chemical composition [2]. The main components of essential oils are monoterpenes and sesquiterpenes, they exert antibacterial effects by structural and functional damage of bacterial cell wall [5]. The known antimicrobial activities of essential oils are based on the ability to destroy the cell wall and cell membranes, they act by causing destabilization of bacterial membranes, damaging the membrane proteins, depleting proton motive force, and releasing cell contents [6]. Essential oils are accumulated mainly by plants as secondary metabolites.

Though, a few of them are found in animals and microorganisms [7]. Essential oils are relatively distributed in the plant kingdom in about 60 plant families that comprise close to 2000 species. The major plant families that yield commercially significant essential oils are found mostly in dicotyledonous plants [8]. However, some monocotyledonous and gymnospermatophyta type of plants also produce the essential oils found in the markets [8, 9]. Some essential oils have also been reported in pteridophytes, bryophytes, algae and fungi too [7].

Plant species of the genus *Hypericum* have a wide scope of secondary metabolites which are responsible for their many biological activities [10]. Scores of research studies have demonstrated that essential oils have chemical compounds with varied biological and pharmacological properties. In the *Hypericum* genus, *Hypericum perforatum* is the most popular and best studied of all the *Hypericum* species across the world and has been found largely useful in mild to moderate depression, neuroprotection, antineuralgic, and antiviral properties [11]. Also, biological activities, such as antibacterial, antioxidant, analgesic, anti-inflammatory, hypotensive and spasmolytic properties have been reported for *Hypericum perforatum* [12]. The other *Hypericum* species have demonstrated antioxidant, antimicrobial, anti-inflammatory, cytotoxic, antimalaria, analgesic, wound and burn healing properties [12, 13] and out of the various biological activity studies of the essential oils from *Hypericum* species, antimicrobial activities is the most reported [13, 14].

The species, *Hypericum revolutum* subsp. *keniense* is used in traditional medicine to treat skin disorders, nervous disorders, gastrointestinal disorders and rheumatism [15, 16]. Limited works on biological, pharmacological and phytochemical studies of *Hypericum revolutum* subsp. *keniense* have been reported for the organic and aqueous extracts, that is antimicrobial, brine shrimp lethality and oral acute toxicity and preliminary phytochemical studies that revealed the presence of saponins, cardiac glycosides, flavonoids, tannins, coumarins, carotenoids and volatile oils [17] and isolation of betulinic acid and coumarins [17, 18]. To best of our knowledge, there are no reports on the biological or pharmacological activities of the essential oils from *Hypericum revolutum* subsp. *keniense*. The objective of the current study was to establishing the antibacterial, antioxidant potency and characterize the composition of the essential oil components from this plant. The findings from this study imperatively provide scientific data to validate the ethnomedicinal claims of using the plant extracts for the management of gastrointestinal, skin and musculoskeletal disorders.

2.0 Material And Methods

2.1 Collection of plant materials

The fresh leaves and flowers of *Hypericum revolutum* subsp. *keniense* were collected from Kieni village, Gakoe sublocation in Kiambu County. The plant was authenticated by a taxonomist at the East Africa Herbarium of the National Museums of Kenya. A voucher specimen number GS001-2020 was prepared and deposited at the Department of Botany in the East Africa Herbarium. The collected leaves and

flowers were air dried at room temperature and pressure for two weeks. The dried plant materials were the ground into course powder using a blender.

2.2 Extraction of essential oil from leaves and flowers of *H. revolutum* subsp. *keniense*

The leaves and flower powders of *H. revolutum* subsp. *keniense* (500 g) were extracted using Clevenger like apparatus for 2 hours according to the British Pharmacopoeia Steam Distillation Method [19]. The essential oils that were extracted, were collected and dried over anhydrous sodium sulphate, thereafter, the oils were weighed and stored in airtight sealed vials at 4 °C until antibacterial and GC-MS analysis of the essential oils were conducted.

2.3 Microorganisms used for antibacterial assay

The microorganisms that were used in this study were American Type culture collections (ATCC) and clinical (Laboratory collections). The ATCC microorganisms were *Staphylococcus aureus* (ATCC 12393), *Escherichia coli* (ATCC 25922), *Acinetobacter baumannii* (ATCC 19606), *Salmonella* Enteritidis (NCTC12023), *Salmonella* Typhimurium (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 15442) were obtained from Kenya Medical Research Institute (KEMRI) laboratory. In addition, *Haemophilus influenzae* (ATCC 49766) and *Pseudomonas aeruginosa* (ATCC 15442) and a clinical strain of methicillin-resistant *Staphylococcus aureus*, were sourced from National Public Health Laboratories Services at Kenyatta Hospital. All bacterial strains were handled and transported aseptically. The bacteria were grown and maintained in small vials containing Nutrient broth at 4 °C using a Samsung refrigerator (Samsung electronics, Seoul, South Korea) until the time of assay.

2.4 Preparation of culture media, plates, inoculum bacterial stains

2.4.1 Mueller Hinton media and plates

The Mueller Hinton Agar (MHA) of lot number 0000378040 and Mueller Hinton broth (MHB) of lot number 0000349752 manufactured by HiMedia Laboratories (India) were prepared according to the manufacturer's instruction [20, 21]. About 20 ml of the partially cooled sterile media (45 °C) was poured onto the sterile disposable petri dishes to make Mueller Hinton plates with uniform media depth of 3 mm. The Mueller Hinton Agar plates had been marks of six equal segments to ensure that a disk was not placed closer than 24 mm (center to center) on the MH agar plate.

2.4.2 Bacteria inoculum

Each of the eight bacteria used in this study were subcultured overnight at 37°C in Mueller-Hinton agar plates. Direct colony suspension method was then used to prepare bacteria inoculum by transferring five morphologically similar colonies from fresh Mueller Hinton Agar plates using a heat sterilized wire loop into about 5 mL of sterile normal saline in a capped test tube and were vortexed. The suspension

containing bacterial inoculum was adjusted using sterile normal saline to obtain a turbidity equivalent to that of a 0.5 McFarland standard BaSO₄ which was prepared spectrophotometrically and therefore an approximate 1.5×10^8 Colony Forming Units per milliliter (CFU /mL) were prepared.

2.4.3 Preparation of resazurin (Alamar blue)

Resazurin was obtained from Monks Medicare Africa Ltd in tablet form. Resazurin solution was prepared according to the manufacturer's specification. A tablet was dissolved in 50 mL of sterilized distilled water and vortex, thereafter a ratio of 1:10 final volume was used for the assay.

2.5 Antibacterial assay

2.5.1 Agar disk diffusion assay

Agar disk diffusion assay was done according to the 12th edition of Clinical and Laboratory Standards Institute (CLSI) protocol in 2015 [22] with minor modifications as described in 2016 by Hudzicki [23]. Each bacteria inoculum of approximate 1.5×10^8 CFU/mL was diluted in a ratio 1: 100 using sterile normal saline to obtain a colony suspension of 1×10^6 CFU/mL in an inoculum tube. Then, a sterile swab was dipped into inoculum tube and the swab was rotated against the side of the tube (above the fluid level) using firm pressure to remove excess fluid and swap dripping wet. Thereafter, the dried surface of a MH agar plate was inoculated by streaking the swab three times over the entire agar surface. The plate was rotated approximately 60 degrees each time to ensure an even distribution of the inoculum. Sterile disks (6 mm in diameter) were impregnated with 10 µl of (100%, 50%, 25% and 12.5% v/v) of the *H. revolutum* subsp. *keniense* leaf and flower essential oils. The impregnated disks were placed on the surface of the agar, using sterilized forceps at a distance of more than 24 mm apart. Experiments for each sample were conducted in triplicates and 10 µl of ciprofloxacin (0.5 mg/ml) was used as positive controls while 10 µl of dimethylsulfoxide (0.1%) was used as a negative control. The inoculated plates containing the essential oil impregnated disks were incubated for 24 hours at 37 °C and respective zones of inhibition were measured in mm using digital zone of inhibition measuring caliper.

2.5.2 Broth microdilution assay

Minimum inhibitory concentration (MIC) of *H. revolutum* subsp. *keniense* essential oils were evaluated by broth microdilution assay according to the procedures described in 2017 by Ohikhena *et al.* [24] and modified in 2020 by Mogana *et al.* [25]. First, Mueller Hinton broth (100 µl) were put into the wells from the second to the eleventh column of row B to G. Secondly, hundred microliters of *H. revolutum* subsp. *keniense* essential oils extracts at concentration 500 µg/mL and the standard drug (ciprofloxacin at concentration 0.8 µg/mL) were added into row B of column 2 to 9 in quadruplicate using multichannel pipette.

Hundred microliters were removed from the starting concentrations (columns 2–9 in row B) and transferred to the next row and properly mixed with the 100 µl broth that was already in the wells. Two-fold serial dilution from row C to G was done by transferring 100 µl from row C to row D systematically,

followed by mixing gently using microchannel pipette. The procedure was repeated up for row (E), (F) and (G). The last 100 µl that was removed from row G was discarded. The 10th and 11th columns were treated as negative control which served as growth control (drug-free) columns. Hundred µl of the diluent (1% DMSO in MH broth) were added into 100 µl in row B of column of the 10th and 11th column. Two - fold serial dilution was done from row B to row G of the 10th and 11th columns. Finally, the resultant volume in all the test wells with the extracts, the standard drugs and the negative controls was 100 µl. A volume 5 µl of the 1×10^6 CFU/ml bacteria inoculum was transferred into all wells except for the wells in the row G which served as blank control (microorganism and drug free).

The microtiter plates were incubated at 37 °C for 18 hours after which, 60 µl of Alamar blue resazurin was added to all the wells and then incubated for 20 minutes after which colour change from blue/purple to pink was observed to indicate minimum inhibitory concentration. The viable cells produce oxidoreductases which convert blue resazurin to resorufin (pink) therefore indicating microbial growth and used to determine minimum inhibitory concentration [25, 26].

2.5.3 Determination of minimum bactericidal concentration

Minimum bactericidal concentrations (MBC) was determined according to the procedure described by Man et al. [27] with slight modifications. Three microliters of Mueller Hinton broth were taken from the last three wells of each row that indicated no bacterial growth and was subcultured in the petri dish plates containing 20 ml fresh Mueller Hinton agar (MHA) and were incubated for 24 h at 37 °C. The lowest concentration without visible microbial biomass growth under optical microscope was defined as MBC, indicating the death of 99.5% of the original inoculum.

2.5.4 Disposal of microbiological waste

The used petri dishes, 96-micro-titre well plated and bacteria inoculum were autoclaved before disposing them into trash.

2.6 Investigation of Antioxidant activity

2.6.1 DPPH (2,2-diphenyl-2-picrylhydrazyl) assay

The antioxidant activity of *Hypericum revolutum* subsp. *keniense* leaf essential oil extract was evaluated using 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay method described by Moriasi *et al.* [28] with slight modifications. Test concentrations of *Hypericum revolutum* subsp. *keniense* leaf essential oil extract and standard (L- ascorbic acid) were prepared at concentrations of 1000 µg/ml, 100 µg/ml, 10 µg/ml, 1 µg/ml, 0.1 µg/ml and 0.01 µg/ml in methanol. DPPH (0.1 mM) was prepared in methanol and 2.4 ml were added to 1.6 ml of the different concentrations of the extract and the standard (L-ascorbic acid). The control for the current study consisted of a mixture of 2.4 ml of (0.1 mM) 2,2-diphenyl-2-picrylhydrazyl (DDPH) solution in methanol and 1.6 ml of methanol. The test samples and the standard were prepared in triplicate test tubes which were then kept in the dark at room temperature for 15 minutes to allow reaction to take place. Absorbances were measured at wavelength of 517 nm against

methanol as the blank using UV spectrophotometer. The test was conducted in triplicates and the percentage radical scavenging activity calculated using the formula:

$$\%RSA = \frac{Abs. Control - Abs. Test}{Abs. Control} \times 100$$

Key: %: percentage, RSA: radical scavenging activity, abs.: absorbance

Antioxidant potency was expressed as a value of concentration of *Hypericum revolutum* subsp. *keniense* leaf essential oil that was responsible for 50% inhibition of DPPH radicals (IC₅₀). IC₅₀ values for the oil and the standard were calculated from the relationship curve of RSA versus concentrations of the respective sample and standard curves. The antioxidant activity levels were classified based on the IC₅₀ values, very strong (IC₅₀ of less than 50 µg/ml), strong (IC₅₀ of between 50 and 100 µg/ml), medium (IC₅₀ of between 100 and 150 µg/ml), weak (IC₅₀ of between 150 and 200 µg/ml) and very weak (IC₅₀ of more than 200 µg/ml) [29].

2.6.2 Ferric reducing antioxidant power (FRAP) Assay

The reducing power of *H. revolutum* subsp. *keniense* essential oil mixture was evaluated by protocol of Moriasi et al. [28] with minor modifications. The essential oil and L-ascorbic acid different concentrations (0.01, 0.1, 1, 10, 100, and 1000 µg/ml) were prepared in methanol. Exactly one-milliliter of the extract or the standard at different concentrations was added into 2.5 ml of 200 mM phosphate buffer (pH 6.6) and 2.5 ml of 30 mM potassium ferricyanide. The resulting mixture was incubated for 20 min at 50 °C.

Thereafter, 2.5 ml of 600 mM trichloroacetic acid (TCA) was added into the cooled reaction mixture and then centrifuged at 3000 rpm for 15 minutes. A volume of 2.5 ml of the supernatant was mixed with an equal volume of distilled water. Finally, 0.5 ml of 600 mM ferric chloride was added to the mixture. The absorbance of both the standard (Ascorbic acid) and the extracts at different concentrations, were measured at a wavelength of 700 nm against the blank using a spectrophotometer (Lab tech model Double beam UV-Vis). The concentrations of the oil and standard that yielded an absorbance value of 0.5 were determined from the graph of absorbance at 700 nm against extract concentrations and considered as the median effective concentration (EC₅₀). Increase in absorbance indicated high antioxidant activity. In each case, the experiment was performed in triplicate.

2.6.3 Hydrogen peroxide radical scavenging capacity Assay

The method of Arika et al. [30] with minor modification was adopted. The *H. revolutum* subsp. *keniense* essential oil and L-ascorbic acid were prepared to yield different concentration in methanol. Hydrogen peroxide solution (40 mM) was prepared using a phosphate buffer (pH 7.4.). One-millilitre of different concentrations of essential oil/standard (1000 – 0.01 µg/ml) was added to 0.6 ml of hydrogen peroxide solution and incubated for 10 min at room temperature. The absorbance of the solutions were measured at 230 nm against a blank consisting of phosphate buffer (pH 7.4) only. All the tests were performed in triplicates and the scavenging activity of hydrogen peroxide was calculated using the formula stipulated by Muhammed et al. [31].

$$\% \text{Hydrogen peroxide scavenging activity} = \left(\frac{AO - A1}{AO} \right) \times 100$$

where *AO* is the absorbance of the control reaction and *A1* is the absorbance in the presence of the samples or standards.

2.7 Chemical analysis of *H. revolutum* subsp. *keniense* essential oil mixtures

2.7.1 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Chemical composition of the essential oil isolated from *H. revolutum* subsp. *keniense* leaves and flowers were analyzed by Gas Chromatography -Mass spectrometry SHIMADZU 2010 plus (GC-MS). The GC-MS analyses were done in three independent repetitions. A 7890A gas chromatography interfaced with mass spectrometer triple quad system fitted with 7693 auto-sampler, Agilent Technologies, and installed with Mass Hunter Workstation Software Version B.05.00 build 5.0.519.0, service pack I © Agilent technologies 2011, for qualitative analysis. A capillary column DB-5, 30 m × 0.25 mm ID and column film thickness of 0.25 µm composed of 100% dimethylpolysiloxane was used. Helium of 99.9999% purity was used as a carrier gas at a flow rate of 1 ml/min. A sample of essential oil was introduced to the injector at a volume of 1 µl and the split ratio of 10: 1. The inlet temperature was maintained at 250°C. The oven temperature was programmed at 110°C for 4 min, then increased to 240°C and finally to 280°C at a rate of 20°C for 5 minutes. The total run time for the extract was 40 minutes. The MS transfer line was maintained at a temperature of 250°C and the source temperature was maintained at 280°C. The mass fragments were analyzed using electron impact ionization at 70 eV, a scan interval of 0.5 seconds and the fragments were read from 45 to 450 Da, and the obtained data was evaluated using total ion count (TIC).

2.7.2 Identification of compounds

The compounds in *H. revolutum* subsp. *keniense* essential oils were identified from chromatograms based on their elution times. The chromatograms of the eluted compounds were deconvoluted and their mass spectra matched with those of the NIST 11 mass spectral database. The NIST 11 database is a fully evaluated collection of electron ionization (EI) mass spectra for various compounds and it contains MS/MS spectra and GC-data of over 243, 893 spectra of 212, 961 unique compounds with identifications, nearly all with chemical structures. Quantifiable data of *H. revolutum* subsp. *keniense* essential oils contents were computed from electronic integration of the Total Ion Chromatogram (TIC) peak areas and recorded as Area percentage of Total which was then consider percentage composition of the oil.

2.7.3 FTIR spectroscopy fingerprinting of *H. revolutum* subsp. *keniense* essential oils

Fourier Transform Infra-Red spectroscopy analysis of the essential oils was done by adopting the method used by Wangia et al. [32]. *H. revolutum* subsp. *keniense* essential oils from the leaves and flowers were mixed with spectroscopic grade potassium bromide (KBr) in the ration of 1:10 by a mortar and pestle. The resultant fine powder mixture was compressed using a hand press (5×10^6 Pa) in an evacuated die to produce a clear transparent disc of 13 mm diameter and 1 mm thick. The disc was placed in a sample handler and was scanned twenty times at the frequency regions of between 4000 to 450 cm^{-1} and the spectra produced were recorded.

2.7.4 Evaluation of total phenolic content (TPC)

Total phenolic content of *Hypericum revolutum* subsp. *keniense* leaf essential oil extract was determined based on Folin-Ciocalteu reagent assay, as described by Saeed et al. [33] with slight modifications. Different concentrations range from 150 to $4.6875\text{ }\mu\text{g/ml}$ of gallic acid (0.3 ml) were prepared and they were mixed with 1.5 ml of Folin-Ciocalteu's phenol reagent (10%) by swirling using test tubes. *Hypericum revolutum* subsp. *keniense* leaf essential oil extract (0.3 ml) were put in a separated test tube and mixed with 1.5 ml Folin-Ciocalteu's phenol reagent (10%) by swirling using test tubes too. Thereafter, 1.5 ml of 7.5% Na_2CO_3 solution were made in deionized water and were mixed with 0.3 ml of different concentrations of gallic acid and *Hypericum revolutum* subsp. *keniense* leaf essential oil extract. The mixtures were incubated for 2 minutes in the dark at room temperature, after which the absorbance was read at 760 nm. The TPC was determined from extrapolation of gallic acid standard calibration curve. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of the sample in dry weight. Results were calculated and expressed as microgram of Gallic acid equivalents (GAE) per gram extract using the formula described by Siddiqui et al. [34].

$$C = \frac{cxv}{m}$$

Where C in mgGAE per gram dry weight of sample, c: concentration of gallic acid ($\mu\text{g/ml}$) extrapolated from the curve equation, v: was the volume in which the sample was diluted (ml) and m, mass of the extract weighed in grams.

2.7.5 Evaluation of total flavonoid content (TFC)

Total flavonoid content (TFC) of *Hypericum revolutum* subsp. *keniense* leaf essential oil extract was determined by Aluminium nitrate colorimetric method described by Cosmulescu, et al. [35] with some modifications. In brief, 0.125 ml of 1% *Hypericum revolutum* subsp. *keniense* leaf essential oil extract in methanol and different concentrations of catechin (0.625 to $20\text{ }\mu\text{g/ml}$) were prepared using test tubes in triplicates. Five percent of 0.075 ml Sodium nitrate (NaNO_3) were added to the test tubes containing the test sample and the different concentrations of the standard (Catechin). Thereafter, the mixture was incubated for six minutes at room temperature. After, 0.15 ml of 10% Aluminium nitrate (AlNO_3) were added to the sample mixture and the mixtures containing different concentrations of the standard, followed by the addition of 0.75 ml of 4% Sodium Hydroxide and the mixtures were made up to 2.5 ml by

distilled water. The absorbance of the reaction mixtures were measured at 510 nm using UV spectrophotometer. The blank was setup by following the same procedure however, the plant extract was replaced with an equal volume of methanol. All the determinations were carried out in triplicate. The total flavonoid content was expressed as milligram of Catechin equivalents (CE) per gram dry weight of extract respectively using the formula described by Siddiqui et al. [34].

$$C = \frac{cxv}{m}$$

Where C in mgCE per gram dry weight of sample, c: concentration of catechin in µg/ml) that was extrapolated from the curve equation, and v: was the volume in which the sample was diluted (ml) and m, mass of the extract weighed in grams.

2.8 Statistical analyses

Antimicrobial and antioxidant results were presented as the mean ± standard error of the mean. The One-Way ANOVA with Turkey's *post hoc* test was performed for the comparison between the means. The statistical analyses were performed using Minitab statistical software version 20 (Minitab, LLC., USA). The data were considered significant when the *P* value was < 0.05.

2.9 Ethical Considerations

Permission to carry out the study and ethical clearance was granted by the office of the Registrar Research and Extension, Kisii University on a letter with Ref: KSU/R&E/03/5/529. The methods for the current study were reviewed by Mount Kenya University Scientific and Ethics Review Committee and a certificate number 875 was granted for research in a letter with Ref: MKU/ERC/1802. The National Commission for Science, Technology and Innovation (NACOSTI) license number NACOSTI/P/21/10472 was also granted for the current research in a letter with reference number 294203.

3.0 Results

3.1 Extraction yield

The dried leaves and flowers had a percentage essential oils yield of 7.5 ± 07% and 3.8 ± 03% respectively. The essential oil mixture obtained from the leaves was yellow in colour with sweet floral aroma while the one from the flower was brown with sweet fruity aroma.

3.2 Antibacterial activity of *H. revolutum* subsp. *keniense* essential oils

In this study, the flower and leaf derived essential oils of *H. revolutum* subsp. *keniense* produced significantly larger growth inhibition zones against the tested bacteria at 100% concentration than at lower concentrations (*P* < 0.05; Table 1). There were no significant statistical differences in growth inhibition of the tested bacteria observed between plates incubated with 12.5% v/v and 25% v/v, and 25%

v/v and 50% v/v of the flower and leaf oils of *H. revolutum* subsp. *keniense* ($P > 0.05$; Table 1). Notably, the inhibition zones produced by the leaf and flower essential oils against methicillin-resistant *S. aureus* and *H. influenzae* and the flower oil against *A. baumannii* and *S. aureus* were not significantly different from those produced by the standard antibiotic (ciprofloxacin 0.05% w/v) ($P > 0.05$; Table 1).

Besides, the inhibition zones produced by the two types of essential oils at each concentration against all the test bacteria, except methicillin-resistant *S. aureus*, were not significantly different ($P > 0.05$; Table 1). On the methicillin-resistant *S. aureus*, the leaf derived essential oil of *H. revolutum* subsp. *keniense* showed a statistically significantly larger inhibition zone than the flower derived essential oil ($P < 0.05$; Table 1); however, the inhibition zones produced by the two oils at all the other concentrations were not significantly ($P > 0.05$) different as shown in Table 1.

Table 1
Antibacterial activity of the flower and leaf oils of *H. revolutum*

Bacterial Strain	EOs extract	Concentration and Inhibition Zone Diameter (mm)				Standard (0.05% w/v)
		12.5%v/v	25%v/v	50%v/v	100%v/v	
MRSA	FL	12.00 ± 0.00 ^b _A	14.00 ± 0.58 ^b _A	14.67 ± 0.67 ^b _B	27.33 ± 0.33 ^a _A	29.70 ± 1.20 ^a _A
	LF	13.00 ± 1.00 ^c _A	15.00 ± 0.58 ^{bc} _A	17.33 ± 0.67 ^b _A	28.00 ± 0.58 ^a _A	29.70 ± 1.20 ^a _A
<i>S. aureus</i> (ATCC 12393)	FL	10.33 ± 0.33 ^d _A	14.00 ± 0.58 ^c _A	15.67 ± 0.33 ^c _A	28.67 ± 0.88 ^b _A	31.61 ± 0.27 ^a _A
	LF	11.67 ± 0.33 ^c _A	15.00 ± 0.58 ^b _A	16.67 ± 0.88 ^b _A	32.00 ± 1.00 ^a _A	31.61 ± 0.27 ^a _A
<i>A. baumannii</i> (ATCC 19606)	FL	12.00 ± 0.00 ^{da} _A	13.33 ± 0.33 ^{cd} _A	14.67 ± 0.33 ^c _A	31.33 ± 0.88 ^b _B	38.30 ± 0.35 ^a _A
	LF	12.00 ± 0.00 ^c _A	13.33 ± 0.33 ^{bc} _A	15.33 ± 0.33 ^b _A	36.67 ± 0.88 ^a _A	38.30 ± 0.35 ^a _A
<i>S. Enteritidis</i> (NCTC12023)	FL	10.67 ± 0.33 ^d _A	11.33 ± 0.33 ^d _A	15.00 ± 0.00 ^c _A	22.67 ± 0.33 ^b _A	30.30 ± 0.69 ^a _A
	LF	11.00 ± 0.58 ^d _A	12.00 ± 0.00 ^d _A	15.33 ± 0.33 ^c _A	25.00 ± 0.58 ^b _A	30.30 ± 0.69 ^a _A
<i>S. Typhimurium</i> (ATCC 14028)	FL	10.00 ± 0.00 ^d _A	12.00 ± 0.58 ^{cd} _A	14.00 ± 0.00 ^c _A	22.33 ± 0.67 ^b _A	28.63 ± 0.60 ^a _A
	LF	10.33 ± 0.33 ^d _A	12.33 ± 0.67 ^{cd} _A	14.00 ± 0.58 ^c _A	23.00 ± 0.58 ^b _A	28.63 ± 0.60 ^a _A
<i>P. aeruginosa</i> (ATCC 15442)	FL	11.00 ± 0.58 ^c _A	12.00 ± 0.00 ^c _A	13.68 ± 0.33 ^c _A	20.67 ± 0.33 ^b _A	30.00 ± 1.50 ^a _A

Values are presented as $x \pm SEM$ for three replicates. Means with dissimilar superscript lower-case alphabets within the same row are significantly different ($P < 0.05$; One-Way ANOVA with Turkey's *post hoc* test), Whereas means with dissimilar subscript uppercase alphabets within the same bacterial strain and concentration are significantly different ($P < 0.05$; independent student t-test statistic). Eos: Essential oils; FL: flower oil extract of *H. revolutum*; LF: Leaf oil extract of *H. revolutum*; MRSA: Methicillin-Resistant *S. aureus*; Standard: Ciprofloxacin (0.05% w/v) and ZI for negative control = 0 mm

	LF	11.33 ± 0.33 ^c _A	12.33 ± 0.33 ^c _A	14.67 ± 0.33 ^c _A	20.67 ± 0.33 ^b _A	30.00 ± 1.50 ^a _A
<i>E. coli</i> (ATCC 25922)	FL	11.00 ± 0.00 ^d _A	11.67 ± 0.33 ^d _A	13.67 ± 0.33 ^c _A	20.67 ± 0.33 ^b _A	39.30 ± 0.35 ^a _A
	LF	11.33 ± 0.33 ^d _A	12.67 ± 0.33 ^{cd} _A	14.00 ± 0.00 ^c _A	21.67 ± 0.33 ^b _A	39.30 ± 0.35 ^a _A
<i>H. influenzae</i> (ATCC 49766)	FL	12.33 ± 0.33 ^c _A	14.33 ± 0.33 ^c _A	19.33 ± 0.67 ^b _A	28.33 ± 0.88 ^a _A	29.00 ± 0.00 ^a _A
	LF	12.67 ± 0.33 ^c _A	14.67 ± 0.33 ^c _A	21.33 ± 0.88 ^b _A	29.33 ± 1.20 ^a _A	29.00 ± 0.00 ^a _A

—

Values are presented as $\bar{x} \pm SEM$ for three replicates. Means with dissimilar superscript lower-case alphabets within the same row are significantly different ($P < 0.05$; One-Way ANOVA with Turkey's *post hoc* test), Whereas means with dissimilar subscript uppercase alphabets within the same bacterial strain and concentration are significantly different ($P < 0.05$; independent student t-test statistic). Eos: Essential oils; FL: flower oil extract of *H. revolutum*; LF: Leaf oil extract of *H. revolutum*; MRSA: Methicillin-Resistant *S. aureus*; Standard: Ciprofloxacin (0.05% w/v) and ZI for negative control = 0 mm

A summary of the lowest concentrations of the *Hypericum* leaf and flower essential oils which prevented visible growth (MIC) of the tested bacteria were tabulated (Table 2). The lowest MIC values that were revealed in this study were 15.625 (µl/ml), this was demonstrated by the essential oils from the leaves of *H. revolutum* subsp. *keniense* against methicillin-resistant *Staphylococcus aureus*, *Salmonella* Enteritidis and *Escherichia coli*. Also, the lowest concentration of the oils that was bactericidal (MBC) was 15.625 (µl/ml) for methicillin-resistant *Staphylococcus aureus*. The MBC:MIC ratio was either one (1) or two (2) throughout the experiment.

Table 2
Showing MIC and MBC of *H. revolutum* subsp. *keniense* leaf and flower essential oils

Microorganism	Plant part	MIC (µl/ml)	MBC (µl/ml)	MBC: MIC Ratio
MRSA	L	15.625	15.625	1
	Fl	250	250	
<i>Staphylococcus aureus</i> (ATCC 12393)	L	125	250	2
	Fl	125	250	
<i>Acinetobacter baumannii</i> (ATTC 19606)	L	62.5	125	2
	Fl	62.5	125	
<i>Salmonella</i> Enteritidis (NCTC12023)	L	15.625	31.25	2
	Fl	31.25	62.5	
<i>Salmonella</i> Typhimurium (ATCC 14028)	L	62.5	125	2
	Fl	125	250	
<i>Pseudomonas aeruginosa</i> (ATCC 15442)	L	15.625	31.25	2
	Fl	62.5	62.5	1
<i>Escherichia coli</i> (ATCC 25922)	L	15.625	31.25	2
	Fl	125	250	
<i>Haemophilus influenzae</i> (ATCC 49766)	L	125	125	1
	Fl	125	125	
Fl; Flower, L; Leaf, MBC; Minimum bactericidal concentration, MIC; minimum inhibitory concentration, MBC: MIC; Minimum bactericidal concentration: minimum inhibitory concentration ratio, MRSA: methicillin-resistant <i>Staphylococcus aureus</i> ; µl/ml; microliter per milliliter				

2.3 Antioxidant activity of *H. revolutum* subsp. *keniense* leaf essential oil mixture

Three *in vitro* antioxidant activity assay methods were used to demonstrate the ability of the *Hypericum* essential oil from *H. revolutum* subsp. *keniense* to scavenge for free radicals. The methods are based on spectrophotometry. An organic radical DPPH, Fe(III) complex and H₂O₂, a Non Free-Radical Species. The percentage radical scavenging activity of the essential oil obtained from *H. revolutum* subsp. *keniense* in the current study revealed IC₅₀ values of the oil as more than 1000 µg/ml for DPPH assay, 0.31 µg/ml for FRAP assay and 12.33 µg/ml in the case of Hydrogen peroxide assay.

2.3.1 Antioxidant activity by DPPH assay

The three methods of assay had variant IC₅₀ values and this is consistent with literature that explain that different methods utilize different principles [36]. On the basis of the description done in 2020 by Minsas et al. [29], the DPPH assay revealed very weak antioxidant activity (IC₅₀ > 200 µg/ml) when compared to that of L- ascorbic acid which was very strong (IC₅₀ < 50 µg/ml). The very high IC₅₀ values in the DPPH method of assay are ascribed to the inadequacy of method to the lipophobic systems and given that the essential oil in this case provides for the nonpolar system [37, 38].

2.3.2 Ferric-Reducing Antioxidant power of the leaf essential oil of *H. revolutum*

The FRAP method, a non-free radical involvement method that focus on the monitoring of the process of reducing of ferric iron (Fe³⁺) to ferrous iron (Fe²⁺). The concentration of the *Hypericum* essential oil under this study was 0–1000 µg/ml and the calculated ferric reducing antioxidant power that converted half of the Fe³⁺ to Fe²⁺ known as IC₅₀ was 0.31 µg/ml. As the results in Table 2 indicate, the absorbances of the reaction mixtures containing the leaf oil of *H. revolutum* subsp. *keniense* at concentrations of 0.32 and 1.6, as well as 8.0 and 40.0 (µg/ml) were not significantly different (P > 0.05); however, these absorbances were significantly lower than those recorded at concentrations of 200.0 and 1000.0 µg/ml (P < 0.05). Furthermore, the standard (L-Ascorbic acid) showed a significant concentration-dependent increase in the measured absorbances (P < 0.05; Table 3). Notably, the differences between the absorbances of the *H. revolutum* subsp. *keniense* leaf oil and L-ascorbic acid recorded at concentrations of 0.32, 200, and 1000 µg/ml were insignificant (P > 0.05); however, at other concentrations, the absorbances recorded by L ascorbic acid were significantly higher than those recorded by the studied oil (P < 0.05; Table 3).

Table 3
 Ferric-Reducing Antioxidant Power of the *H. revolutum* leaf essential oils

Concentration (µg/ml)	Absorbance (700 nm)	
	HRLEOs	L-ASA
0.32	0.5247 ± 0.0035 ^c _A	0.5942 ± 0.0176 ^e _A
1.60	0.6065 ± 0.0077 ^c _B	0.7008 ± 0.0141 ^d _A
8.00	0.6225 ± 0.0059 ^c _B	0.8509 ± 0.0060 ^c _A
40.00	0.6684 ± 0.0188 ^c _B	0.8747 ± 0.0068 ^{bc} _A
200.00	0.9622 ± 0.0406 ^b _A	0.9189 ± 0.0090 ^{ab} _A
1000.00	1.1569 ± 0.0687 ^a _A	0.9635 ± 0.0162 ^a _A

Values are presented as $\bar{x} \pm SEM$ for three replicates. Means with different superscript lower-case alphabets within the same column are significantly different ($P < 0.05$; One-Way ANOVA with Turkey's *post hoc* test), Whereas means with different subscript uppercase alphabets within the same row are significantly different ($P < 0.05$; independent student t-test statistic). HRLEOs: *H. revolutum* leaf essential oils; L-ASA: L-ascorbic acid.

2.3.3 Hydrogen peroxide scavenging activity

The percentage hydrogen peroxide scavenging activity of the *H. revolutum* subsp. *keniense* leaf oil increased significantly ($P < 0.05$) in a concentration-dependent manner, as shown in Table 4. However, the percentage hydrogen peroxide scavenging activity of L-ascorbic acid recorded at between concentrations of 40.0 and 200.0 µg/ml, 1.6 and 8.0, and 0.32 and 1.6 µg/ml respectively, were not significantly different ($P > 0.05$); whereas that recorded at a concentration of 1000.0 was significantly higher than those recorded at all the other concentrations ($P < 0.05$; Table 4). Notably, the leaf oil of *H. revolutum* subsp. *keniense* had a significantly higher percentage of hydrogen peroxide scavenging activity than L-ascorbic acid at all concentrations ($P < 0.05$; Table 4). The ability of *H. revolutum* subsp. *keniense* leaf essential oil to scavenge for hydrogen peroxide (H₂O₂) was studied. The IC₅₀ value was calculated to be 12.33 µg/ml, which indicated such concentration was required to quench half of the available H₂O₂ molecules, therefore offering protection from tissue damage [32, 39].

Table 4
Hydrogen Peroxide Scavenging Activity of the *H. revolutum* leaf oil

Concentration (µg/ml)	HRLEOs	L-ASA
0.32	20.74 ± 0.86 ^e _A	10.18 ± 0.36 ^d _B
1.60	38.17 ± 0.56 ^d _A	12.93 ± 0.26 ^{cd} _B
8.00	43.77 ± 0.77 ^d _A	14.97 ± 0.57 ^c _B
40.00	61.54 ± 0.79 ^c _A	21.26 ± 1.30 ^b _B
200.00	72.09 ± 3.56 ^b _A	22.59 ± 0.47 ^b _B
1000.00	85.42 ± 1.52 ^a _A	75.24 ± 1.08 ^a _B

Values are presented as $\bar{x} \pm SEM$ for three replicates. Means with different superscript lower-case alphabets within the same column are significantly different ($P < 0.05$; One-Way ANOVA with Turkey's *post hoc* test), Whereas means with different subscript uppercase alphabets within the same row are significantly different ($P < 0.05$; independent student t-test statistic). HRLEOs: *H. revolutum* leaf essential oils; L-ASA: L-ascorbic acid.

3.3 Chemical composition of *H. revolutum* subsp. *keniense* essential oils

3.3.1 Chemical composition of essential oils obtained from *H. revolutum* subsp. *keniense* leaves (HRLEO) and flowers (HRFEO)

The results obtained from the current study showed that *H. revolutum* subsp. *keniense* essential oils contained complex mixtures of more than 100 compounds (Table 5). The major components of the essential oils that were revealed in the GC-MS spectra of the essential oil mixture that was isolated from the leaves were Caryophyllene (22.1%), α -Farnesene (9.2%), 1-Octyl trifluoroacetate (7.9%), 2-ethyl-2-methyl-Oxirane (6.3%), (Z,E)-3,7,11-Trimethyl-1,3,6,10-dodecatetraene (4.5%), α -Copaene (4.3%), 3-(2-Bromoethyl)dihydro-2(3H)-furanone (3.1%), α -Acetobutyrolactone (2.8%), trans-1,2-bis-(1-methylethenyl)cyclobutene (2.3%), cis-Calamenene (2.2%), α -Gurjunene (1.8%), Caryophyllene oxide (1.8%) and Cadina-1(6),4-diene (1.5%). Different percentage of essential oil composition was observed in the essential oil mixture that was derived from flowers and the main chemical components were 7-(1-Methylethylidene)bicyclo(4.1.0)heptane (15%), (E)-2,7-dimethyl-3-Octen-5-yne (11%), 2,3,4-trimethylhexane (10.5%), 2-ethyl-2-methyl-Oxirane (8.8%), Caryophyllene (8.5%), 1-Octyl trifluoroacetate (7.9%), 4,4-Dimethyloxazolidine (6.6%), D-Camphene (5.3%), α -Copaene (3.2%), 2-cyclopropylpentane (1.6%), β -Ocimene (1.5%) and Undecane (1.5%).

Table 5

Chemical composition of essential oils obtained from *H. revolutum* subsp. *keniense* leaves (HRLEO) and flowers (HRFEO)

No.	RT (Min)	Compound	% Content of Total	
			HRLEO	HRFEO
1	1.7	2-methyl-Butane	0.1	0.1
2	1.71	N-Dimethylaminomethyl-tert.-butyl- isopropylphosphine	0.2	0
3	1.72	N,N-Dimethyl-3-buten-1-amine	0.3	0
4	1.9	4,4-Dimethyloxazolidine	0	6.6
5	2.0	3-(2-Bromoethyl)dihydro-2(3H)-furanone	3.1	0
6	2.02	α . -Acetobutyrolactone	2.8	0
7	2.1	2-ethyl-2-methyl- Oxirane	6.3	8.8
8	2.3	1-Octyl trifluoroacetate	7	7.9
9	2.5	2-chloro- Hexane	1.3	0
10	2.5	2-cyclopropylpentane	0	1.6
11	5.4	5-methyl-2-(1-methylethyl)- 1-Hexanol	0	0.1
12	6.3	Nonane	0.9	0
13	6.4	2,3,4-trimethylhexane	0	10.5
14	7.3	α . -Pinene	2.3	0
15	7.4	(E)- 2,7-dimethyl-3-Octen-5-yne	0	11
16	7.8	L-Camphene	0	0.4
17	7.9	2,4-Thujadiene	0	0.1
18	8.6	β -Pinene	0.2	0
19	8.7	D-Camphene	0	5.3
20	8.8	β -Myrcene	0.1	0.6
21	9.0	Decane	0	0.1
22	9.4	Thujene	0	0.1
23	9.7	α -Terpinene	0	0.3

Key: HRLEO; *H. revolutum* essential oil, HRFEO; *H. revolutum* essential oil, RT; Retention time in minutes, % Content of Total; mean percentages composition of the oils from triplicate experiments. 0% Content of Total; any value of less than 0.1

No.	RT (Min)	Compound	% Content of Total	
			HRLEO	HRFEO
24	10.2	trans-1,2-bis-(1-methylethenyl)cyclobutane	2.3	0
25	10.3	7-(1-Methylethylidene)bicyclo(4.1.0)heptane	0	15
26	10.6	β -Ocimene	0.1	1.5
27	11.0	γ -Terpinene	0.1	0.4
28	11.9	3-Methyl-6-(1-methylethylidene)-cyclohexene	0.1	0.4
29	12.1	Undecane	0.1	1.5
30	12.2	3-Nonanol	0	0.1
31	13.1	exo-Fenchol	0	0.1
32	14.5	2-Pentadecyn-1-ol	0	0.1
33	15	Pinocamphone	0	0.1
34	15.5	L- α -Terpineol	0	0.1
35	15.6	Myrtenal	0	0.1
36	19.7	α - Cubebene	0.2	0
37	20.2	α - bulnesene	0.1	0.1
38	20.4	1,2,3,4,4a,7-Hexahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene	0	0.2
39	20.41	Cyclosativene	0	0.2
40	20.43	α -ylangene	0.3	0.4
41	20.6	α - Copaene	4.3	3.2
42	21.5	α -Gurjunene	0.8	0.5
43	22.1	Caryophyllene	22.1	8.5
44	22.2	β -copaene	0.9	0.1
45	22.4	epi- β -Caryophyllene	1.4	1
46	22.5	Selina-5,11-diene	0	0.1
47	22.6	(1R,4aS,8aR)-1,4a-Dimethyl-7-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	0.1	0

Key: HRLEO; H. revolutum essential oil, HRFEO; H. revolutum essential oil, RT; Retention time in minutes, % Content of Total; mean percentages composition of the oils from triplicate experiments. 0% Content of Total; any value of less than 0.1

No.	RT (Min)	Compound	% Content of Total	
			HRLEO	HRFEO
48	23.0	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	0.6	0
49	23.4	1S,4aR,8aS)-1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene	1.4	0
50	22.9	(1Z,4Z,7Z)-1,5,9,9-tetramethylcycloundeca-1,4,7-triene	0.8	0.3
51	23.2	Cadina-1(6),4-diene	1.5	0
52	23.3	γ-Amorphene	0	1.2
53	23.4	(1S,4aR,8aS)-1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene	1.2	0.7
54	23.6	(Z,E)-3,7,11-Trimethyl-1,3,6,10-dodecatetraene	4.5	0.1
55	23.7	δ-Selinene	0	0.1
56	23.8	[1aR-(1aα,7α,7aβ,7bα)]-1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-1H-cycloprop[e]azulene	0.9	0
57	23.8	guaia-1(10) 11-diene	0	0.7
58	24.0	α-Farnesene	9.2	0.7
59	24.7	cis-Calamenene	2.2	0
60	24.8	11-Hydroxy-11-methyl-tricyclo [4.3.1.1(2,5)] undecan-10-one	0.1	0
61	24.9	Cubenene	1	0
62	24.92	α-Amorphene	0	0.2
63	25.0	(4aR,8aS)-4a-Methyl-1-methylene-7-(propan-2-ylidene) decahydronaphthalene	0	0.2
64	25.04	(1S,4aR,8aS)-1-Isopropyl-4,7-dimethyl-1,2,4a,5,8,8a-hexahydronaphthalene	0.2	0.2
65	25.1	9-Methoxycalamenene	0	0.3
66	25.13	α-Maaliene	0	0.3
67	25.2	α-Calacorene	0.3	0

Key: HRLEO; H. revolutum essential oil, HRFEO; H. revolutum essential oil, RT; Retention time in minutes, % Content of Total; mean percentages composition of the oils from triplicate experiments. 0% Content of Total; any value of less than 0.1

No.	RT (Min)	Compound	% Content of Total	
			HRLEO	HRFEO
68	25.5	(E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	1.4	0.2
69	25.6	Ledene oxide-(II)	0	0.1
70	25.62	cis-(-)-2,4a,5,6,9a-Hexahydro 3,5,5,9-tetramethyl(1H) benzocycloheptene	0	0.1
71	25.64	Isoaromadendrene epoxide	0.1	0
72	25.7	(1aR,4S,4aR,7R,7aS,7bS)-1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol	0.1	0.1
73	26.0	1,1,4,7-Tetramethyldecahydro-4aH-cyclopropa[e]azulen-4a-ol	0.2	0.2
74	26.3	(5S,6R,7S,10R)-7-Isopropyl-2,10-dimethylspiro[4.5]dec-1-en-6-ol	0.9	0
75	26.4	Caryophyllene oxide	1.8	0.6
76	26.5	5-Azulenemethanol	0.3	0
77	26.6	[1aR-(1a α ,4 β ,4a β ,7 α ,7a β ,7b α)]-decahydro-1,1,4,7-tetramethyl-1H-cycloprop[e]azulen-4-ol	0.2	0.2
78	26.7	Bicyclosesquiphellandrene	0.1	0
79	26.8	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	0.1	0
80	26.83	Ledol	0	0.1
81	26.9	Globulol	0.6	0.2
82	2.70	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	0.2	0
83	27.1	2-(bromomethyl)- 2-adamantanol	0.1	0
84	27.4	Di-epi-1,10-cubenol	1.8	0
85	27.5	β -Guaiene	0.3	0
86	27.6	10-epi- γ -Eudesmol	0.5	0
87	27.62	Cryptomeridiol	0.1	0

Key: HRLEO; H. revolutum essential oil, HRFEO; H. revolutum essential oil, RT; Retention time in minutes, % Content of Total; mean percentages composition of the oils from triplicate experiments. 0% Content of Total; any value of less than 0.1

No.	RT (Min)	Compound	% Content of Total	
			HRLEO	HRFEO
88	27.7	Epicubenol	0.9	0.4
89	27.73	τ -Cadinol	0.8	0.2
90	27.8	α -muurolol	0.3	0.2
91	27.9	Cyclocopacamphan-12-yl methyl ether A	0.1	0
92	28.0	Isoaromadendrene epoxide	0	0.1
93	28.1	τ -Muurolol	0.4	0
94	28.13	2-(4a,8-dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl)propan-2-ol	0.3	0
95	28.2	Bulnesol	0.2	0
96	28.4	trans-Z- α - Bisabolene epoxide	0.3	0
97	28.42	Heptadecane	0	0.1
98	28.6	8-Isopropyl-1,5-dimethyltricyclo [4.4.0.02,7] dec-4-en-3-one	0.2	0
99	28.9	(1R,7S, E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	0.2	0
100	29.1	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	0.1	0
101	29.3	trans-Valerenyl acetate	0.1	0
102	29.5	3-(1-Methoxy-2-methyl-2-propanyl)-1,2,4,5-tetramethylbenzene	0.1	0
103	29.8	(-)-Spathulenol	0.3	0
104	30.1	3,4,4a,5,6,7-hexahydro-2H-naphthalen-1-one	0.1	0
105	32.4	di-2-methylpropyl phthalate	0.1	0
106	32.8	2,6,10,15-Tetramethylheptadecane	0	0.3
107	36.8	Heneicosane	0	0.1
108	37.2	Phytol	0.1	0

Key: HRLEO; H. revolutum essential oil, HRFEO; H. revolutum essential oil, RT; Retention time in minutes, % Content of Total; mean percentages composition of the oils from triplicate experiments. 0% Content of Total; any value of less than 0.1

3.3.2 Fourier Transform Infra-red spectra of *H. revolutum* subsp. *keniense* essential oils

The Fourier Transform Infra-red spectra that were obtained from the current study revealed eighteen and seventeen peak values that were interpreted to represent different functional groups. FTIR spectral analysis was used to identify the functional groups of the secondary metabolites that are associated with medicinal values. The region above 1200 cm^{-1} revealed eleven and nine spectral peaks due to the vibration of individual bonds or functional groups of the compounds that were in the essential oils that were obtained from *Hypericum revolutum* subsp. *keniense* leaves and flowers respectively (Table 6 and Figs. 1 and 2). On the other hand, six spectral peaks were observed in the region below 1200 cm^{-1} , which is known as the 'fingerprint region' that indicates bands due to the vibrations of the complete bioactive molecule. The fingerprint region was notable with many infrared bands. The bands represent different vibrations, including those of O-H, -CH (CH₂), C = O, C = C-C, C-H and C-N single bond stretches.

Table 6
Assignment of FTIR frequencies of *H. revolutum* subsp. *keniense* essential oils

Peak number	Frequency peak value of <i>H. revolutum</i> subsp. <i>keniense</i> essential oils		Assignment of functional group
	HRLEO	HRFEO	
1	3749.4	Not detected	O-H stretch
2	3315.5-3160.2	3417-3255.6	O-H stretch
3	2923	2923	A Symmetric stretching of –CH (CH ₂) vibration
4	2862.2	2862.2	Symmetric stretching of –CH (CH ₂) vibration
5	2731.8	Not detected	O-H stretch
6	2376.1-2113.8	2376.1-2275.8	C-H bend
7	1635.5	1643.2	C = C, Stretch
8	1558.4	1566.1	Carboxylate
9	1450.4	1450.4	C-H bend
10	1380.9	1380.9	O-H bend, Alcoholic group
11	1257.5	1257.5	CN, Aromatic primary amine
12	1180.4	1180.4	C-O
13	1103.2	1110.9	CH ₃
14	987.5	987.5	C-H Alkene
15	887.2	887.2	C-H
16	748.3	740.6	-(CH ₂) _n

3.3.4 Phenolic contents of *H. revolutum* subsp. *keniense* leaf essential oil extract

Phenolic content of the *Hypericum* oil isolated from the leaves of *H. revolutum* subsp. *keniense* was measured using the Folin–Ciocalteu reagent. The results were derived from a calibration curve ($Y = 0.009x + 0.1158$, $R^2 = 0.9975$) of gallic acid (0–150 µg/ml) and expressed in gallic acid equivalents (GAE) per gram dry extract weight (Fig. 3.). The total phenolic content in the essential oils derived from the leaves was calculated and found to be 130.4 ± 10.5 mg GAE/g dry weight.

3.3.5 Flavonoid content of *H. revolutum* subsp. *keniense* leaf essential oil extract

The quantitative determination of flavonoids in the essential oils derived from the leaves of total leaves of *H. revolutum* subsp. *keniense* was done by Aluminium nitrate colorimetric method. The results were derived from the calibration curve ($Y = 0.0208x + 0.0149$, $R^2 = 0.952$) of catechin (0.625–20 µg/ml) (Fig. 4.) and expressed as mg Catechin equivalent (CE) per gram of essential oils dry weight. The calculations revealed that the total flavonoid content essential oil from the dried leaves of *H. revolutum* subsp. *keniense* was 0.911 ± 0.04 mg CE/g dry weight.

4.0 Discussion

The current study provides a report on the antibacterial, antioxidant and chemical composition of *H. revolutum* subsp. *keniense* essential oil mixture obtained from leaves and flowers for the first time. However, the essential oils from other *Hypericum* species have been reported to have activity against a range of bacteria [40, 41]. The antibacterial activity of essential oils extracts from *H. revolutum* subsp. *keniense* leaves and flowers were interpreted according to De Almeida Alves et al. [42] as very high antibacterial activity at 100% undiluted oil. The essential oils demonstrated broad spectrum antibacterial activity against both gram-positive and gram-negative bacteria. The MIC values of both leaves and flowers essential oils extracts less than 8000 µL/ml, and can be interpreted according to Voukeng et al. [43] as significant antibacterial activity. The MBC/MIC ratio was found to less than 4 (< 4) for the *H. revolutum* sub species *keniense* leaf and flowers oils extracts against all the assayed bacteria in this study. Therefore, it was interpreted to mean that the both the oils had bactericidal effects [25]. More so, the antioxidant activities of the *Hypericum* oil from *H. keniense* subsp. *keniense* in the current study are consistent with the literature of the essential oils from other *Hypericum* species, more especially the exceptionally and highly studied *Hypericum perforatum* [48, 49].

A considerable number of chemical constituents in the essential oils detected in this study were consistent with the ones found in other *Hypericum* species in literature, though it was noted that quantities of the composition were different from those of other *Hypericum* species. Some of the major compounds that were identified in the essential oils derived from *Hypericum* leaves and flowers have been reported previously by Crockett and Schepetkin et al. [40, 44], caryophyllene, caryophyllene oxide, ocimene, pipene, spathulenol, myrcene, amorphene, selinene and undecane were reported in the current study of the essential oils *H. revolutum* subsp. *keniense*. However, germacrene D, and limonene which are commonly identified in the essential oils of other *Hypericum* species, were not identified. The chemical content and percentage variations in the components of essential oils. The variations that were observed in the *Hypericum* oil in the current study are attributed to environmental factors, plant parts, harvesting time, drying procedures and storage conditions as described by Bergonzi et al. [47]. The antibacterial and antioxidant activities of some of the major compounds of *H. keniense* subsp. *keniense* leaf

or flower. Caryophyllene and α -pinene is indicated to have antibacterial properties in literature [51– 54]. D- Camphene is also known for its antioxidant activities [48].

The interpretation of FTIR spectra that sixteen bands provide information of varied classes of chemical components of the essential oil. The main classes being alcohols, phenolics, carbonyl ketones, amides and aldehydes. The band at 3749.4 cm^{-1} for the leave essential oil, indicated the non-bonded O-H stretch of the hydroxyl group, the broad band between $3315.5\text{--}3160.2\text{ cm}^{-1}$ and $3417\text{--}3255.6\text{ cm}^{-1}$ for the leaf and flower essential oils respectively depicts the O-H of the hydroxyl group of alcohols. Both the leaf and flower essential oils FTIR spectra revealed the bands at 2923 cm^{-1} and 2862.2 cm^{-1} which are characteristic of asymmetric and symmetric stretch vibrations of C-H groups. Also, the band at 2731.8 represented the C-H group of aldehydes [52]. C-H group of the essential oils was confirmed by the presence of the spectra band at 1450.4 cm^{-1} and 987.5 cm^{-1} Burman et al. [53]. The band at 1635.5 cm^{-1} and 1643.2 cm^{-1} in the FTIR the spectra of the leaf and flower derived essential oils respectively, was characteristic of the presence of C = C which absorbs at $1680 - 1620\text{ cm}^{-1}$ [54]. The presence of C = C band on the FTIR spectra was associated to the presence compounds with olefinic unsaturated group. In this case an example is farnesene [55]. The bands at 1558.4 cm^{-1} and 1566.1 cm^{-1} of the leaf and flower essential oil spectra respectively represent the presence of stretch vibrations that were indicative of compounds containing carboxylate or carboxylic acid salt that is proposed to originate from 1-Octyl trifluoroacetate [56]. The two peaks FTIR spectrum of the *H. revolutum* subsp. *eniense* essential oils that appear at ~ 1450.4 and at $\sim 1380.9\text{ cm}^{-1}$ originate possibly from caryophyllene due to the presence of isopropyl and gem-dimethyl groups which give rise to a split umbrella mode [50, 51].

The plant extracts that contain phenolic and flavonoid molecules are known to have antibacterial and antioxidant properties [59]. The antibacterial potency of the phenolic and flavonoid compounds is attributed to their ability to destroy the membrane of bacteria, inhibit the bacterial virulence factors such as enzymes and toxins, and suppress bacterial biofilm formation [60]. On the other hand, these molecules donate hydrogen to neutralizes and deactivate the free radicals, and have therefore gained popularity for preventing diseases caused by oxidative stress over the synthetic antioxidants [56, 57]. The available literature indicates that some group of compounds in the essential oils of plants are classified as terpenes, straight-chain compounds not containing any side chain, phenylpropanoids and miscellaneous group having varied structures not included in first three groups (sulfur or nitrogen-containing compounds). Compounds from these groups have antibacterial and antioxidant potency plants extracts that have phenolic and flavonoid molecules, inhibit bacterial growth as well as preventing cellular damage the action of free radicals [33, 59, 60, 61]. The extracts and essential oils from *Hypericum* species have demonstrated compounds with antibacterial and antioxidant properties [41, 44, 45, 62]. However, there is no study that has been reported over the essential oils revived from the leaves or flowers of *H. revolutum* subsp. *eniense*.

5.0 Conclusions And Recommendation

Based on the results from the current study it was concluded that the essential oils obtained from the leaves and flowers of *Hypericum revolutum* subsp. *keniense* had significant antibacterial activity against methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Pseudomonas aeruginosa*, *Escherichia coli* and *Haemophilus influenzae*. The findings for antibacterial activity are consistent with the traditional uses of *H. revolutum* subsp. *keniense* in folklore medicine in the treatment of diarrhoea, burns and wounds. Based on the FRAP and H₂O₂ methods of antioxidant assay, the essential of the leaves have compounds with antioxidant properties. It was presumed that the antibacterial antioxidant potency was due to the compounds like caryophyllene, α -pinene and D-Camphene. It was recommended that research on antioxidant activities and quantification of phenolics and flavonoids be carried out using the flower derived essential oils. Further efforts are needed to assess the wound healing properties and toxicological profiles of the *H. revolutum* subsp. *keniense* essential oils.

Declarations

Contribution of authors: All authors conceived and designed the research work. Sengera G.O. and Onyancha J. M. performed the experiments, collected, analyzed and interpreted the data. Kenanda E.O and Onyancha J.M supervised the work while Sengera G. O. drafted the manuscript. All authors reviewed, revised read and agreed on the published version of the manuscript.

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Data Availability: All data underlying the results are available as part of the article and no additional source data are required.

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Figures

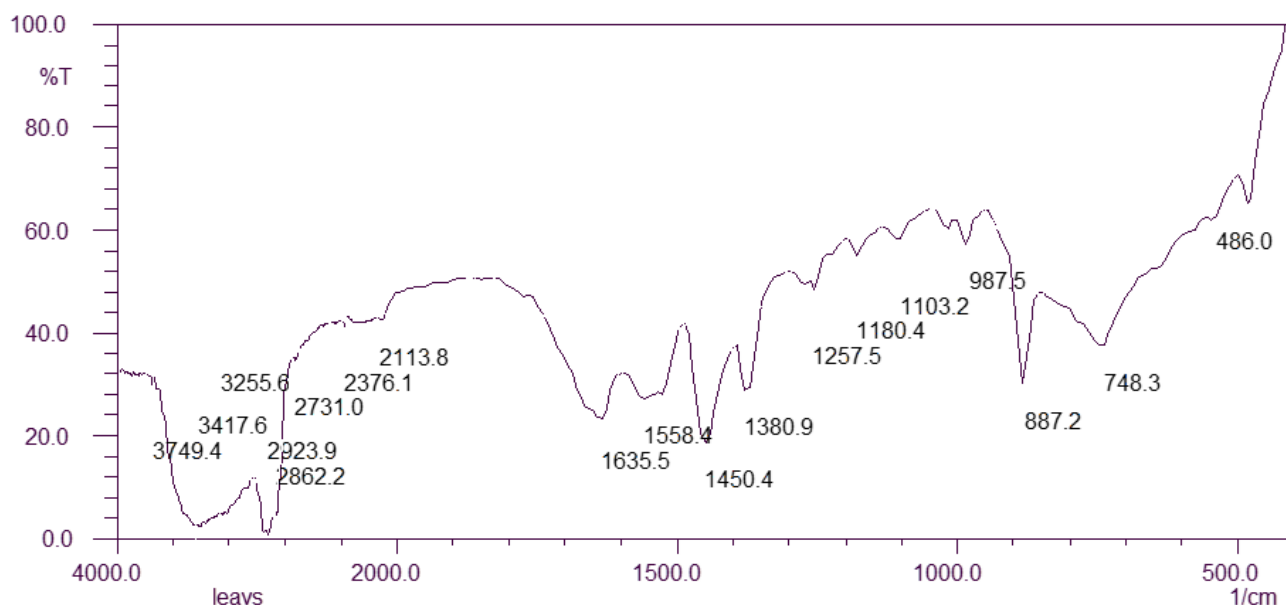


Figure 1

FTIR spectrum of *H. revolutum* subsp. *keniense* leaves essential oil mixture

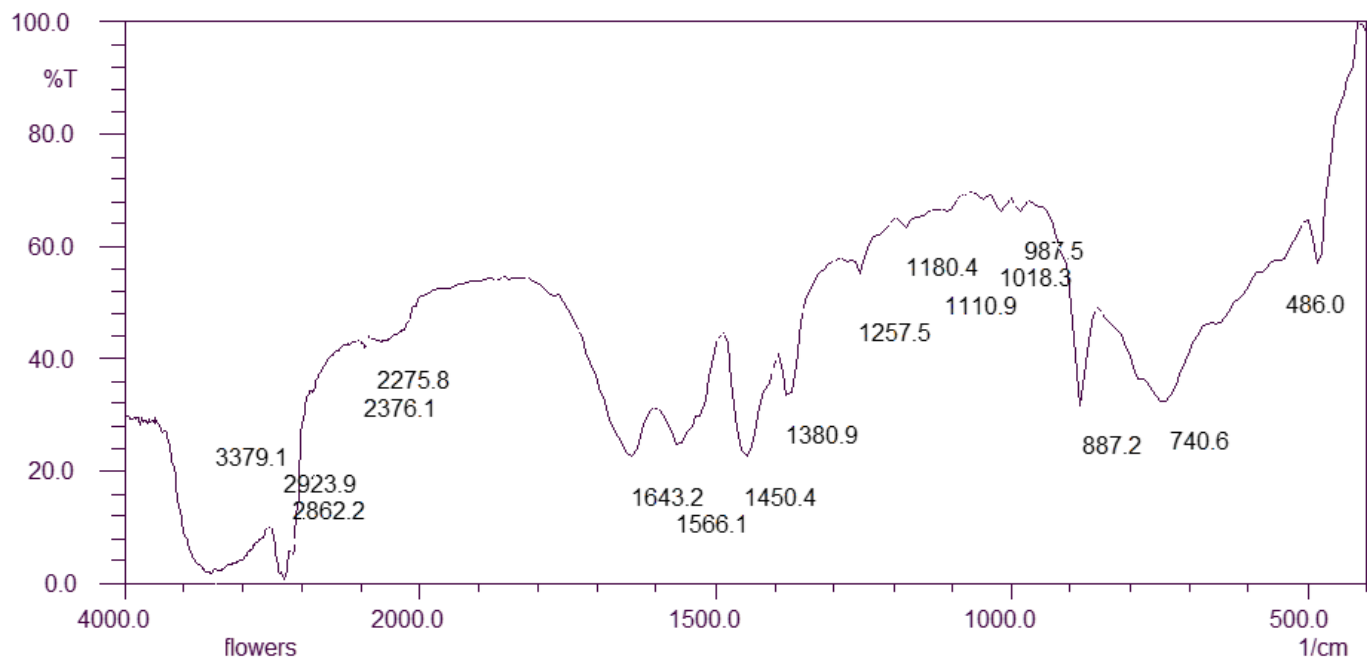


Figure 2

FTIR spectrum of *H. revolutum* subsp. *keniense* flowers essential oil mixture

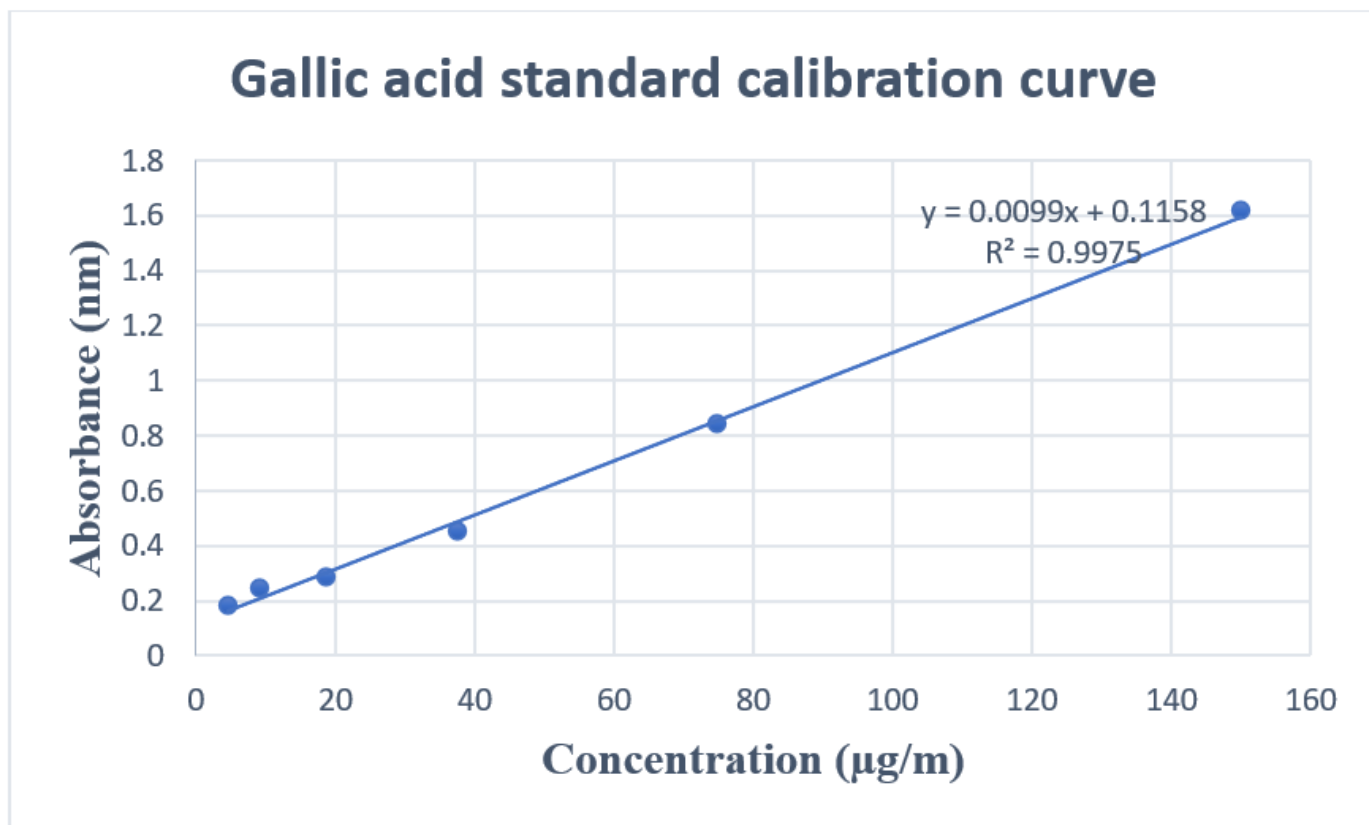


Figure 3

Standard curve of Gallic acid

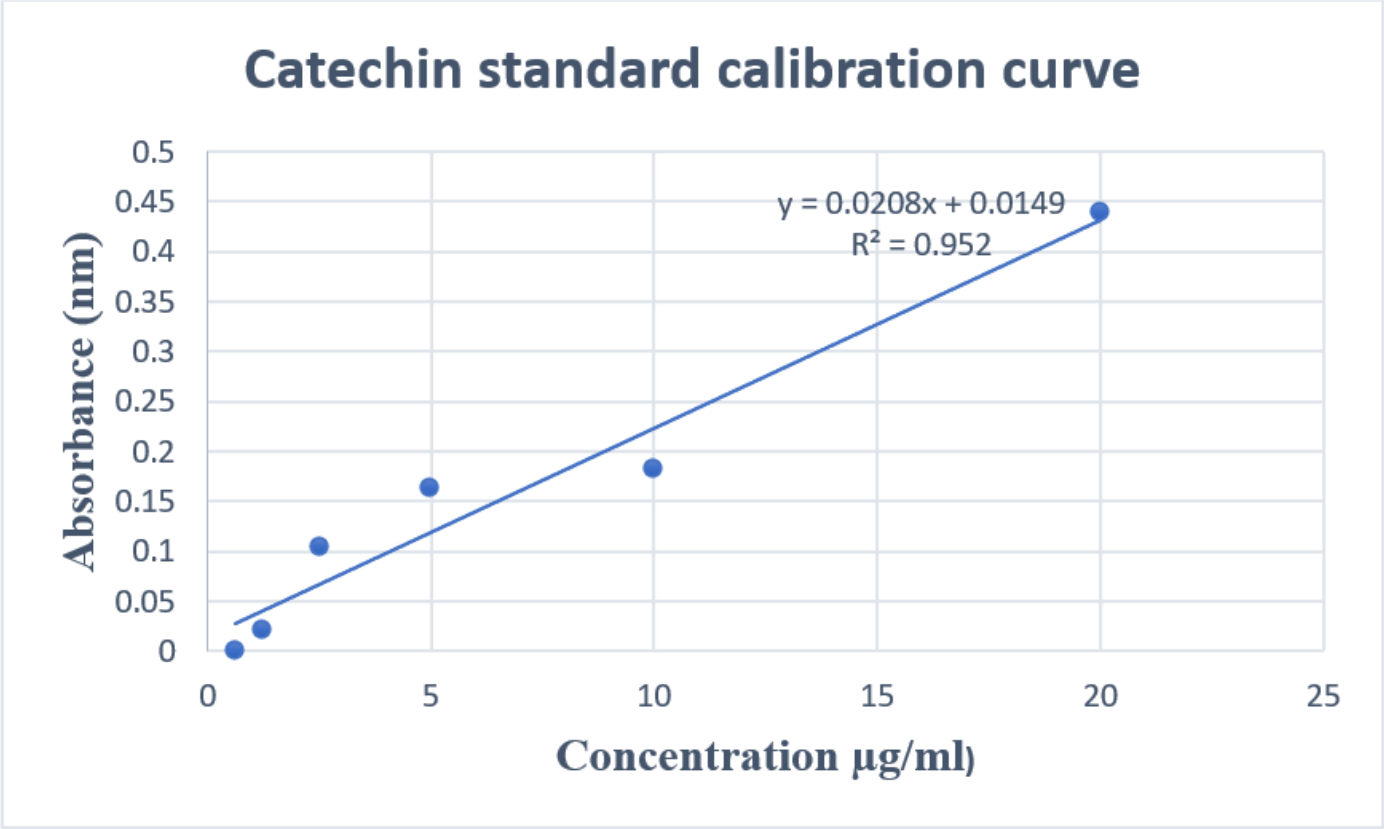


Figure 4

Standard curve of Catechin