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In vitro Antimicrobial Activities, Qualitative Phytochemical Screening, and Free Radical Scavenging Capacities of *Gymnanthemum myrianthum*

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ABSTRACT

Various members of the genus *Vernonia* are commonly used in traditional medicine to treat fever, malaria, diarrhoea, dysentery, hepatitis, cough, fertility inducer and as a laxative. *Vernonia myrianthum* (*Gymnanthemum myrianthum*) is one of the medicinal plants used by traditional healers in Uasin-Gishu and Elgeiyo Marakwet counties to treat communicable and non-communicable diseases such as diabetes mellitus. The objectives of this study were to evaluate the phytochemicals, free radical scavenging capacity and antimicrobial activities of the methanolic root extract of *Vernonia myrianthum*. Standard phytochemical screening methods were employed to qualitatively evaluate the classes of phytochemicals present in the plant. The anti-oxidant capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The disc diffusion method and minimum inhibitory concentration (MIC) were used to screen for antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. *Vernonia myrianthum* was found to contain secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, carotenoids, and coumarin. The antioxidant activity on the extract as based on its IC_{50} values indicated good antioxidant activity. There was no significant difference ($p \geq 0.01$) between the zones of inhibition of *Vernonia myrianthum* and those of those of the positive control gentamycin (GEN, at 10 mcg) and amoxicillin (AMC, 30 mcg) at various test concentrations demonstrating that *V. myrianthum* could inhibit the growth of *E. coli* and *S. aureus*. The findings of this study are critical in understanding and documenting the phytochemistry, the antimicrobial properties and the antioxidant capabilities of *V. myrianthum* thus, laying the groundwork for future research on isolating bioactive compounds from these plants, as well as building the body of evidence needed to justify the ethnomedicinal usage of this plant in the treatment of numerous diseases by different communities.

Keywords: Anti-microbial, antioxidant, *in vitro*, phytochemistry, screening, *V. myrianthum.*

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I. Introduction

Currently, researchers are undertaking efforts to uncover new sources of natural therapeutics which are believed to have fewer side effects and less impact on the aquatic environment Traditional medicine serves around 80% of the world's population's primary healthcare requirements [1]. Therapeutic plants generate a variety of bioactive chemicals with different medicinal properties, including antioxidant, anticancer, anti-inflammatory, antibacterial, antiviral properties among other biological activities [2]. Plant-derived pharmaceutical items have been discovered to have less adverse effects in general, making them an important component of traditional medicines, particularly in rural areas [3]. Phytochemicals originating from plants can thus be used in the drug discovery and development processes [4].

Despite the availability of a significant number of synthetic medications for the treatment of bacterial and fungal infections, the emergence of resistance strains continues to jeopardize the use of such drugs in the treatment of these diseases [5]. These compounds are linked to substantial adverse effects, including generation of increased reactive oxygen species (ROS) in the human body and even mortality, in addition to the challenge of antimicrobial resistance [2]. Kenya has a diverse range of medicinal plants that can be studied for a variety of biological functions in an attempt to find hints and leads that can be developed into new drugs [6].

The emergence of resistant bacterial strains continues to jeopardize the use of currently available medications to treat diseases caused by bacteria [5]. The majority of existing agents have a number of dangerous adverse effects including an increase in reactive oxygen species (ROS) in the human body and even death [2]. As a result, novel ways to circumvent bacterial antibiotic resistance are needed [7], some of which may be the discovery of newer antibiotics from traditional medicinal plants in many African countries

that remain largely unexploited [8].

Antimicrobial resistance (AMR) is a global problem, and the need to contain and control it is globally acknowledged [6]. Africa provides a new frontier for the discovery of new medicinal products, and it has been observed that not much research has been carried out on traditional medicinal plants in many African countries [8]. Despite the fact that certain Kenyan communities continue to utilize the plant identified in this study for therapeutic purposes, there is a scarcity of scientific evidence to back up their efficacy claims [8]. The medicinal plant employed in this study was chosen based on claims made by traditional medicine practitioners in Kenya and other countries about their therapeutic properties [8], [9]. The main aim of this research is to evaluate the antimicrobial activities**,** qualitative phytochemical screening and free radical scavenging capacities of *Vernonia myrianthum*.

II. Methodology

A. Chemicals, Materials and Equipment

All chemicals and solvents used in this study were of analytical grade. The commercially available Nutrient Agar (NA), Muller Hinton Agar (MHA), were used in the antibacterial study.

B. Sample Collection

The roots of *Vernonia myriantha* (Asteraceae) were collected on January 2021 in Uasin-Gishu County (0°30'51.65, 35°16'10.96"E) The plant parts were collected utilizing ethnopharmacological data from the literature and, in certain cases, traditional healer information from Kenya's Rift Valley. The plant's roots were pulled out and washed using distilled water. A trained taxonomist from Kabarak University/University of Eldoret authenticated the plant. The sample was air-dried at room temperature, then reduced to a fine powder with an electric grinder and stored in airtight containers until the extraction process commenced.

The experiment on antimicrobial work and antioxidant activities was set up in a factorial configuration and reproduced three times using Complete Randomized Design (CRD).

C. Preparation of Crude Methanolic Organic Extracts

The extracts were prepared according to the protocol reported by [10], with slight modifications. To make crude methanolic extract, methanol was used in the extraction process. The powdered roots (100 g) of the sample were macerated for 3 days at room temperature in 500 mL of methanol. The filtrate was collected by passing the mixture through Whatman No.1 filter paper using a Buchner funnel. The same procedure was repeated three times to extract all of the chemicals from the insoluble cellular marc (residue), after which the supernatants was mixed. The solvent was evaporated using a rotary evaporator. The percent extractive values for methanol were obtained and reported using the formula:

$$Percentage\ Yield = \frac{Mass\ of\ fraction}{Mass\ of\ plant\ material} \times 100$$

The solid extracts were maintained at 4 °C until use in subsequent experiments.

D. The Qualitative Analyses of the Phytochemicals Present in the Methanolic Root/Tubers Extract

The crude extract was screened to determine the following classes of compounds, with tests carried out using various methods [11].

1) Test for Alkaloids

Twenty grams of powdered extract was dissolved in 8 milliliters of methanol and filtered through Whatman (No 1) filter paper. 2 ml of the extract was divided into two test tubes, and concentrated sulfuric acid was added. Each test tube was added a few drops of Drangendorff's reagent, and was shaken properly. The presence of alkaloids was indicated by the presence of an orange yellow tint.

2) Test for Phenols

Three drops of 5 % ferric chloride solution were added to 2 ml of the extract and mixed before being allowed to settle. The presence of phenolic compounds was indicated by the production of a dark green to blue tint.

3) Test for Terpenoids

A few drops of strong sulfuric acid were added to 2 ml of the extract in chloroform. The mixture was thoroughly agitated before being placed aside for a few minutes. The presence of terpenoids was indicated by a reddish-brown color

4) Test for Saponins

The 2 ml of the extract was mixed with 4 ml of distilled water, agitated, and set aside for a few minutes. The presence of saponins was indicated by the appearance of foaming.

5) Test for Glycosides

The 2 mL of the extract, 2 mL chloroform, and 2 mL concentrated sulfuric acid was mixed and gently agitated, then allowed to settle before making observations. The presence of glycosides was indicated by a red-brown tint.

6) Test for tannins

To 1 mL of filtrate sample and 1 mL of distilled water was combined. 2 drops of ferric chloride were added, and observations was made. The presence of tannins was indicated by a brief greenish to black tint.

7) Test for flavonoids

To 10 mL of ethyl acetate was added 0.5 grams of crude extract and heated for three minutes in a steam bath. This combination was filtered, and the filtrate (4 mL) was combined with 1 mL of ammonia solution and observed. The presence of flavonoids was indicated by the formation of a strong yellow colour.

8) Test for steroids

The crude extract (1 mg) was placed in a test tube and dissolved in chloroform (10 mL), followed by the addition of concentrated sulphuric acid by sides. The presence of steroids was shown by the upper layer in the test tube turning red and the sulphuric acid layer turning yellow with green fluorescence.

E. The Evaluation the Free Radical Scavenging Capacities

Following conventional techniques, the ability of the crude

methanolic extract to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed [12]. The absorbance was read from the spectrophotometer at 518nm and used to calculate the scavenging activity;

Thus

$$Scavenging\ activity = \frac{A518 - A518(blank) \times 100}{A518(blank)}$$

F. The Evaluation of the in vitro Antimicrobial Activities

The crude extract (1 mg) was placed in a test tube and dissolved in chloroform (10 mL), followed by the addition of concentrated sulphuric acid by sides. The presence of steroids was shown by the upper layer in the test tube turning red and the sulphuric acid layer turning yellow with green fluorescence.

1) Preparation of Standard Microorganism's Suspensions

The test organisms (*Escherichia coli, Staphylococcus aureus,*) of the bacterial strains were provided by Kabarak University, Microbiology Department and were cultured according to the procedure by [13]. The test organisms were authenticated and kept in Muller Hinton agar medium in 10 mL Nutrient broth at 37 °C. Muller-Hinton agar was produced as directed by the manufacturer, autoclaved, and dispensed on sterile plates [13].

2) Analysis of antimicrobial activity of extracts

Agar disc diffusion was used to test the antibacterial activity of plant extract, as described by [13], [14]. Each bacterium (*Escherichia coli, Staphylococcus aureus*) received 0.1 mL of broth culture inoculation. 20 mL Muller-Hinton Agar (MHA) was placed into Petri plates and allowed to harden and dry. With a medical cotton swap, bacterial culture was swabbed over the surface of MHA and left to dry for 15 minutes.

In a separate solvent, the plant extract was deposited into sterile 6-mm test discs. Another disc was impregnated with tetracycline as a positive control, and another was impregnated with 40 mL sterile distilled water as a negative control. The test discs were then be placed one by one on the agar surface. The plates were allowed 30-60 minutes at room temperature to allow the extract and controls to diffuse to the agar. Thereafter, the plates were incubated at 37 °C for 24 hours. Inhibition zones (zones with no microbial growth) around the discs was measured and recorded after the incubation period.

G. Data Collection and Analysis

The data was subjected to analysis of variances (ANOVA) with R software at 5% confidence level. When there was a significant difference between the means, Tukey's test for multiple comparison tests at $p \geq 0.05$ was used.

III. Results And Discussion

A. Phytochemical Analysis

The *V. myrianthum* methanolic extracts was tested for various phytochemicals (Table I). Phenols, terpenoids and steroids were determined to be present in abundant concentrations. The phytochemical found to be present in moderate concentrations include alkaloids while those found in trace amounts tannins, flavonoids and saponins, while glycosides were absent.

TABLE I: Phytochemical Analysis of *V. myrianthum* Root Methanolic Extracts

Plants Phytochemicals	Test
Alkaloids	++
Glycosides	-
Phenols	+++
Terpenoids	+++
Tannins	+
Flavonoids	+
Steroids	+++
Saponins	+

+ Traces of phytochemical; ++ Moderate phytochemical concentrations and +++ Abundant phytochemical concentration.

There is limited information regarding the phytochemical spectrum of *V. myrianthum* documentation in literature [15]. reported the presence of alkaloids, flavonoids, saponins, tannins, phenols and coumarins and in a related species namely *Vernonia amygdalina.* In another study by [16], a related species *V. glabra* was reported to contain alkaloids, sapogenins, terpenoids, quinones, and flavonoids. in the crude extracts of (flower, leaf, and root). These results seem to agree well with our findings since the plants are classified under the same genus.

B. Antioxidant Activities

V. myrianthum was tested for free radical scavenging activity using DPPH assay with ascorbic acid as positive control. The absorbance of unscavenged DPPH was found to be 1.159 and was taken as the blank value in all the antioxidant evaluation experiments. A plot of percentage DPPH inhibition versus concentration of ascorbic acid (Fig. 2) depicts a linear relationship implying a dose-dependent free radical scavenging capacity of ascorbic acid.

A plot of percentage DPPH inhibition versus concentration of *V. myrianthum* (Fig. 2) also gave a linear relationship implying a dose-dependent free radical scavenging capacity with an IC_{50} of 0.35 mg/ml (350 ppm).

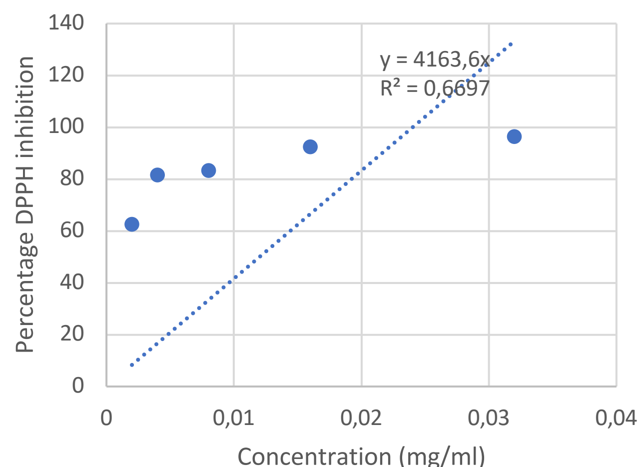

Fig. 1. Graph of percentage DPPH inhibition against the concentration of ascorbic acid.

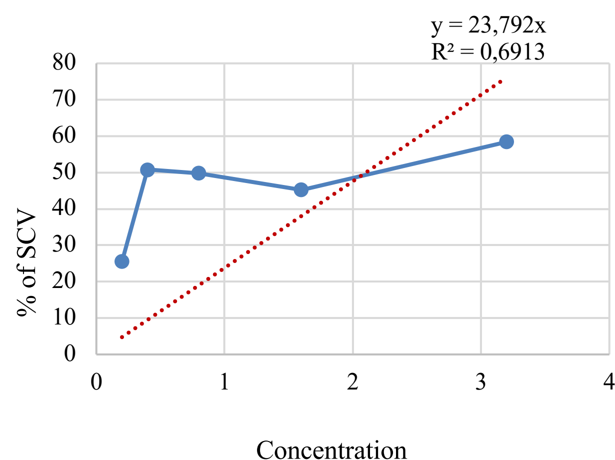

Fig. 2. DPPH radical scavenging activity of V. myrianthum.

The antioxidant capacity of *V. myrianthum* as depicted by the DPPH inhibition IC_{50} value of 0.35 mg/ml (350 ppm) was found to be lower than that of ascorbic acid with a value of 0.00495 mg/ml (1.3 ppm) quoted by [17]. *V. myrianthum* seems to exhibited significant free radical scavenging and antioxidant activities in comparison to the related species *Vernonia amygdalina* which has been found to display a DPPH inhibition IC_{50} value of 1.831 ± 0.15 mg/ml [18]. The ability of this plant extract to scavenge free radicals is attributable to the phenols, terpenoids and steroids found to be present in the root extracts of *V. myrianthum* in significant amounts.

C. *In vitro Antimicrobial Assays*

The methanolic root extract of *V. myrianthum* was tested for *in vitro* antimicrobial assays against *E. coli* and *S. aureus* using disc diffusion method and their means of zones of inhibition obtained Various concentrations (in mg/ml) of *V. myrianthum* displayed dose-dependent activities against both *E. coli* and *S. aureus* (Fig. 3).

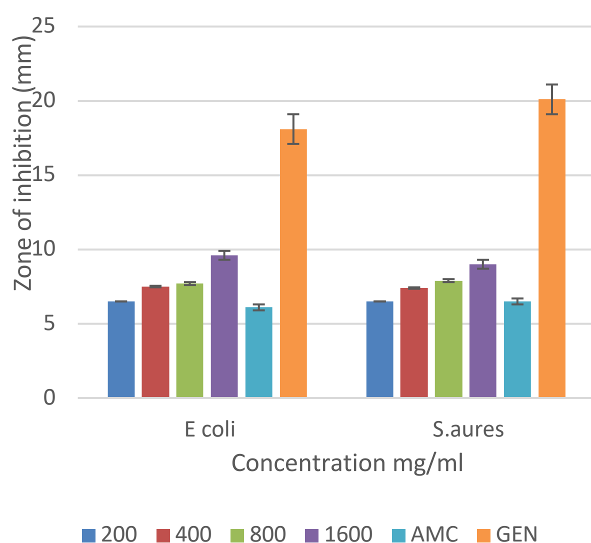

Fig. 1. Graph of percentage inhibition against concentration for *V. myrianthum.*

All the test concentrations of methanolic extracts displayed lower zones of inhibition compared to gentamycin (GEN; 10 mcg). Generally, all the test concentrations of *V. myrianthum* which ranged from 200 to 1600 mg/ml displayed superior zones of inhibition compared to amoxicillin (AMC; 30 mcg). The data for evaluation of minimum inhibitory concentration (MIC) obtained is in (Table II).

TABLE II: MIC VALUES *V. MYRIANTHUM* ROOT FOR METHANOLIC EXTRACTS

	Concentration (mg/ml)	*S. aureus*	*E. coli*
T1	1500	clear	clear
T2	750	clear	clear
T3	375	clear	clear
T4	187.5	clear	clear
T5	93.75	turbid	turbid
T6	46.875	turbid	turbid

IV. RECOMMENDATION

Further studies on *V. myrianthum* are needed to isolate and characterize the exact active component, which are responsible for the antioxidant and antimicrobial activities.

V. CONCLUSION

The plant extract had almost similar phytochemical composition especially flavonoids and phenolics. Root methanolic extract of *V. myrianthum* (IC_{50} value 350 ppm) showed low capacity to scavenge free radicals (IC_{50} value 20 ppm). Root methanolic extract of *V. myrianthum* also dispayed activity against *E. coli* and *S. aureus* (MIC values 187.5 mg/ml each).

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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