

Phytochemical Profiles of *Rhoicissus Tridentata* Harvested From the Slopes Elgon Sub-region, Uganda

Ali Kudamba (✉ alikudamba@gmail.com)

Makerere University

Josephine N. Kasolo

Makerere University

Godfrey S. Bbosa

Makerere University

Allan Lugaajju

Makerere University

Henry Wabinga

Makerere University

Nixon Niyonzima

Uganda Cancer Institute

Moses Ocan

Makerere University

Ali M. Damani

Makerere University

Hussein M. Kafeero

Islamic University in Uganda

Jamilu E. Ssenku

Islamic University in Uganda

Shaban A. Okurut

Islamic University in Uganda

Muniiru Lubowa

Islamic University in Uganda

Abdul Walusansa AW

Islamic University in Uganda

Haruna Muwonge

Makerere University

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Abstract

Introduction: Prostate cancer remains a formidable threat in Uganda. As such, the quest to find novel, efficacious therapies with reduced side effects to avert this challenge is still ongoing. Medicinal plants are taking center stage in these efforts. In the Elgon sub-region of Eastern Uganda, *Rhoicissus tridentata* (L.f.) is one of the medicinal plants commonly used in the management of prostate cancer among communities and traditional herbalists but its phytochemical profiles responsible for toxicity activity have not fully been investigated. This impedes the potential usefulness of *R. tridentata* in developing improved anticancer biotherapeutics. to find novel, efficacious therapies with reduced side effects to avert this challenge is still ongoing. The current study investigated the phytochemical compositions of aqueous and methanol extracts of *Rhoicissus tridentata* used in the management of prostate harvested from the Elgon sub-region.

Methods: The root tubers were harvested from the mountain Elgon sub-region, identified and authenticated, cleaned, air-dried, pounded, and subjected to qualitative phytochemical profiling and spectrophotometry. The laboratory experiments were conducted at Islamic University in Uganda, Uganda Natural Chemotherapeutics Laboratories, and Makerere University. The data were analyzed using descriptive statistics with STATA version 15.0 software. Graphs were plotted using GraphPad Prism® version 9.0.0.

Results: The researchers found that *Rhoicissus tridentata* (L.f.) root tuber extracts were very rich in a number of phytochemical compounds that dissolve better in methanol than in ethanol. Polyphenols, tannins, glycosides, carotenoids, and flavonoids were significantly higher in methanol than in ethanol and water ($p=0.0022$), while saponins were substantially higher in water ($p=0.0022$).

Conclusions: Root tubers of *Rhoicissus tridentata* (L.f.) had several bioactive compounds, with the majority found in methanol as compared to water extract. The bioactive compounds observed were mainly polyphenols and flavonoids, and these have been reported to possess reputable anticancer properties. To help the pharmaceutical industry develop novel and more potent anticancer medications from plants such as *R. tridentata*, it is necessary to undertake in vitro and in vivo investigations on the plant extracts and to purify and characterize the most bioactive compounds.

Introduction

Prostate cancer is the second most common and fifth major cause of cancer-related deaths globally, with 1.4 million cases and 375,000 deaths, reported respectively [1, 2, 3, 4]. Prostate cancer cases are on increase in Uganda at a rate of 5.2% annually causing a significant long term disability among the infected individual and even death [5]. Several prostate treatments are available, including surgery, radiation, chemotherapy, hormone therapy, photodynamic therapy, targeted therapy, immunotherapy, stem cell or bone transplants, and gene therapy [11, 9&10], but their accessibility, cost and associated adverse side-effect remains a challenge among infected individual. However, conventional treatments has been associated with various drawbacks, such as toxicity to both cancer and healthy cells, severe side effects, the appearance of secondary malignancies, being expensive and less widely available, and being ineffective in treating advanced cancer [8]. These negative effects have forced people all over the world to

turn to herbal remedies including Uganda. In Uganda, herbal medicine have continued to be utilized in treatment of illnesses, including cancer[2, 9].

There are several medicinal plant species have been reported to have anticancer activities, including *Allium sativa* L., *Albizia coriaria* (Welw. Ex. Oliver), *Annona muricata* L., *Artemisia annua* L., *Beta vulgaris*, *Rhoicissus tridentata* (L.f.) Wild, and R.B. Drumm[7, 8] and many others. Residents in the Elgon sub-region have for a long-time exhibited the history of using various plant species to treat several malignancies, including prostate cancer. *Tylosema fassoglenisis*, *Hydnora abyssinica* A. Br., *Rhoicissus tridentata* (L.f.) Wild & R.B. Drumm, *Hydnora africana*, and *Albizia coriaria* are among the plant species highly believed to have cancer-healing benefits. Prostate-specific antigen (PSA) observation showed a remarkable reduction in patients who rely on *Rhoicissus tridentata* (L.f.) Wild and R.B. Drumm with respect to all the plants known in this area. However, the phytochemical components of this plant collected from the fertile volcanic soils of Elgon are not fully articulated to the scientific community.

Rhoicissus tridentata (L.f.) Wild and R.B. Drumm belong to the Vitaceae family, which is made up of 16 genera with over 950 species[9]. Members of this family provides a variety of therapeutic benefits but with limited information in regard to prostate cancer treatment[12]. Furthermore, *Rhoicissus digitata*, *Rhoicissus rhomboidei*, and *Rhoicissus tridentata* are known to treat burns, gastrointestinal issues, anti-inflammatory, anticancer potential, backache, body and febrile pain, swelling, malaria, broken bones, menorrhagia, renal complaints, sprained ankles, cuts, epilepsy, sore antiemetic in children, and general pain relief in many parts of the world. *Rhoicissus* plant species are valuable in medicine because they have significant pharmacological potential [11&12] that are utilized as a remedy of diseases in local communities. These species' pharmacological potential are due to a variety of secondary metabolites, they have-including alkaloids, terpenoids, and flavonoids[13]. Gallic acid extracted from *Rhoicissus tridentata* and proanthocyanin monomers and dimers have been demonstrated to stimulate smooth muscle in the rat uterus, which may help with safe delivery[12]. However, the phytochemical compounds in these plants differs amongst plant species and even within the same plant species, plant habitat circumstances, the portions of the plant employed, the solvent used in the extraction process, and the harvesting season[14]. Aqueous and ethanol extracts of *Tylosema fassoglensis*, *Azadirachta indica*, and *Albizia coriaria*, gathered in four separate places in Uganda, were reported to contain large amounts of polyphenols, flavonoids, tannins, and alkaloids in earlier research[8, 13]. However, research is still needed to determine the precise phytochemical makeup of *Rhoicissus tridentata* (L.f.) Wild and R.B. Drumm grown on fertile soils in the Elgon subregion, which remain unclear and leave room for investigation. In the current study, the phytochemical compounds present in *Rhoicissus tridentata* (L.f.) Wild and R.B. Drumm in methanol and aqueous extracts was profiled.

Materials and Methods

Study Design: Experimental study design

Study Area

The study was conducted in two rural districts of Sironko and Bulambuli districts in the Elgon sub-region of eastern Uganda and are thought to have a range of plant species[15]. The distances between Sironko and Bulambuli districts and Mbale city and Kampala, the capital of Uganda are 24.7 km and 55.4 km, and 275.9 km and 306.8 km, respectively[23,24]. Sironko district is bordered to the north by the Bulambuli district, the northeast by the Kapchorwa and Kween districts, the east by Kenya, the southeast by the Bududa district, and the southwest by the Mbale and Bukedea districts. To the north, east, and west of Bulambuli district are Nakapiripirit, Kapchorwa, and Bukedea districts respectively. (Figure 1). The average elevation of these districts is 3996 feet (1,218 meters)[23,24]. According to [18], the average annual temperature is 24.4 °C, while there is between 920 and 1650 millimeters of rain per year on average [18]. The study was conducted in seven villages located at latitudes and longitudes indicated for each as Zema (1.212°E, 34.31°N), Suguta (1.24°N, 34.37°E), and Jewa (1.284°N, 34.31°E) in the Bulambuli district, Nakidoba (1.26° N, 34.49° E), Bulwala (1.25° N, 34.43° E), Miwu (1.21°N, 34.34° E), and Madaya (1.21°N, 34.37° E) in Sironko districts. The majority of the custodians of traditional herbal knowledge were elderly, ranging in age from 55 to 75, and they claimed to have been residents of this region for their entire lives. They have accumulated a broad range of ecological knowledge about the different medicinal plants, as shown by this, over time. However, because they are older than the recommended age range (31–45 years), this puts the sector's sustainability in danger, and grave concerns about collapse continue to be raised[13,21]. As a result, an ethnobotanical study was carried out between September and November 2022 in seven villages, including Zema, Suguta, and Jewa in the Bulambuli district and Nakidoba, Bulwala, Miwu, and Madaya. (Figure 1). The selection of these study sites was shaped under the guidance of local leaders and elders, who, to the best of their knowledge, believed they had the most experienced herbalists.

Climate

The Mount Elgon sub-region primarily experiences a tropical humidity-type climate. A seasonal oscillation of dry northwesterly and moist southwesterly air streams is defined[21]. About 23 °C is the average yearly temperature. Additionally, the average minimum and maximum temperatures are 15 °C. The rainy season starts around March and ends in December[22]. The weak bimodal pattern of mean annual precipitation in the Elgon sub-region is that January through March are the hottest months of the year, while July and August are the coolest. The annual average precipitation is about 1500 mm. In the Mount Elgon sub-region, there is a faint bimodal pattern in the precipitation. Orographic factors and attitude are the main causes of rainfall variations[21].

Soils

The soil structure of Mount Elgon is typically deep and composed of volcanic ash as a result of a single weathering cycle[23]. One significant characteristic stated is that the carbonatite dome's structure makes the soils in this area very variable[24]. The soil types of Mount Elgon have three major sources, according to research[25]. In the beginning, the presence of volcanic ash and agglomerates beneath volcanic ranges, hills, and pediments contributed to the formation of the soils. Rocks that have undergone metamorphism serve as representations of the degraded Gondwanan surface, where some of the soil comes from. Thirdly, another percentage of soil comes from mixed volcanic-metamorphic rocks

Demographic characteristics

The Bagisu, also known as the Bamasaba, make up the majority of the population in this sub-region and are the seventh-largest ethnic group in Uganda [26]. The majority of the locals are peasants who cultivate a variety of multi-season vegetable crops as well as crops like maize, coffee, potatoes, banana (matoke). The region is also reported for having subpar healthcare facilities, primarily Health Centers III and II, as well as a general dearth of institutions specifically designed to treat cancer cases. This makes therapy less accessible, expensive, and unaffordable for a culture that is predominately rural due to its distance from the Uganda Cancer Institute. Therefore, majority of the communities rely and utilize herbal medicine to meet their healthcare needs since they have trouble getting access to standard prostate cancer treatments and because most of their places are isolated and hard to reach areas especially during rainy seasons. Observations of prostate cancer patients who rely on *Rhoicissus tridentata* revealed a significant reduction in PSA with respect to other plants and the need to avail of the phytochemical constituents responsible for these anticancer potentials. This sparked curiosity on understanding plant phytochemical composition in these medicinal plants used in the region in the management of prostate cancer.

Experiment design

Collection of Plant Materials

Plant materials for the experimental investigation were collected from the slopes of the Egon sub-region in January and February 2023, notably from the Sironko and Bulambuli districts from a total of seven villages (Figure 1).

Processing of the Plant Material

The plant materials were cleaned, cut into small pieces, and air-dried until constant weight was obtained in the Biology Laboratory at the Islamic University in Uganda. Then, they underwent qualitative and quantitative examinations at the Natural Chemotherapeutic Laboratory and the Chemistry Department of Makerere University, respectively. The materials were dried and then processed into a coarse powder using a wood powder blender (Model BRONICA).

Extraction Process

The serial extraction approach, with already been established methods by [22,23 with modifications, were adopted. The coarse powder was first soaked in methanol and water as solvents in a series of extraction techniques. The plant rhizomes were gathered, cleaned, let air dry, and then ground into a coarse powder. To enable the extraction of active chemicals, exactly 150g of the powdered materials were weighed using a digital scale (Model I1C28 RAGWAG) and then steeped in 1500 mL of diethyl ether (petroleum ether) in an Ehlmeier flask for 72 hour [29]. Every two hours during the procedure, the mixture was shaken to facilitate the extraction process during the day. It was concentrated at 40 °C and left there to cool until the following day. Using a freezer dryer, the aqueous extract was concentrated. Both the methanol and aqueous extract

yields were calculated. For the duration of the study, all of the samples were maintained in the refrigerator after being sealed in airtight containers.

Determination of the Phytochemical Composition

Qualitative Tests

These tests were conducted following the procedure laid down by Kuruppu et al [31] and Farooq et al [33], with modifications. Some of the chemicals that were tested were phenolics, flavonoids, coumarins, terpenoids, tannins, saponins, phlobatannins, anthraquinone, anthocyanins, steroids and sterols, alkaloids, glycosides, cardiac glycosides, phytosterols, cholesterol, proteins and amino acids, carbohydrates, terpenoids, triterpenoids, diterpenoids, fixed oils and fats, volatile oils, and carotenoids. **Test procedure**

Test for Phenol: Ferric chloride test: To 2 mL of plant extract, a few drops of a 5% ferric chloride solution were added, and the appearance of a dark green or blue-black color indicated the presence of phenol.

Test for Tannins: Braymer's test: 1 mL of the extract was mixed with 3 mL of water and heated in a water bath. The mixture was filtered, and a 10% ferric chloride solution was added to the filtrate. A dark-green color indicates the presence of tannins.

Test for Phlobatannins: HCl test: 2 mL of extract was added to 2 mL of 1% HCl and boiled. The appearance of a red precipitate shows the presence of phlobatannins.

Test for alkaloids: iodine test: 3 mL of the extracts were dissolved individually in dilute hydrochloric acid and filtered. To the filtrate, 3 drops of iodine were added. The appearance of a blue color that disappears on boiling and reappears on cooling shows the presence of alkaloids.

Test of saponins: Foam test: 0.5 g of extract was shaken with 2 mL of distilled water. Formation of frothing (appearance of creamy masses of small bubbles showed presence of saponins)

Test for flavonoids: alkaline reagent test 1 mL of extract was added to 2 mL of a 2% sodium hydroxide solution. An intense yellow color that becomes colorless with the addition of dilute acid signifies the presence of flavonoids.

Tests for anthraquinone: Bontrager's test: 10 mL of 10% ammonia solution was added to a few of the filtrates (shake vigorously for 30 seconds). A pink, violet, or red-colored solution indicates that anthraquinone is present.

Test for anthocyanins: HCl test: To 2 mL of extract, 2 M HCl was added (plus a few drops of ammonia). The appearance of the pink-red solution turning blue-violet after the addition of ammonia showed that anthocyanins are present.

Test for terpenoids: 0.5 g of the extracts was added to 2 mL of chloroform and evaporated to dryness. 3 mL of concentrated sulfuric acid was added. The appearance of a reddish-brown color in the interphase will indicate the presence of terpenoids.

Test for Triterpenoids: Salkowski test: To the 2 mL of extract were added a few drops of concentrated sulfuric acid (shaken well and allowed to stand). The appearance of a golden yellow layer (at the bottom) signifies the presence of triterpenoid.

Test for diterpenes: Copper acetate test: To 2 mL of extract, 4 drops of copper acetate solution were added. The appearance of the emerald green color shows that diterpenoids are present.

Mix 5 mg of extract with 2 mL of chloroform, and then add the same amount of concentrated sulfuric acid along the sides of the test tube. This is how you test for steroids and sterols. Once the upper layer turns red and the lower layer turns yellow with green fluorescence, it indicates the presence of the steroids and sterol compounds in the extract.

Test for Quinones: HCl test: 1 mL of extract was added to a few drops of concentrated HCl. The appearance of the green color shows that quinones are present.

Test for fixed oils and fats: Spot test or stain test: A few little quantities between the filter papers. The observation of oil stains on the paper shows that fixed oils are present.

Test for volatile oils: Fluorescence test: The extract was applied to the filter paper. A transparent appearance (oils and resins) showed that volatile oils are present.

Test for Glycosides: Bontrager's Test 2 mL of hydrolysate was added to 3 mL of chloroform, shake well until the chloroform layer separated, and then add 10% ammonia. The appearance of a pink-colored solution indicates the presence of glycosides.

Test for Cardiac Glycosides: Keller-Killani test: 1 mL of extract, 1.5 mL of glacial acetic acid, 1 drop of ferric chloride solutions, and finally concentrated sulfuric acid (alongside the test tube). A blue-colored solution in the acetic acid layer signifies that cardiac glycosides are present.

Test for Coumarins: Sodium hydroxide test: 2 mL of the plant extract was added to 10% NaOH and then chloroform. The appearance of the yellow color shows that coumarins are present.

Test for proteins and amino acids: Millon's test: 2 mL of the extract was added to a few drops of Millon's reagents. The formation of white precipitates reveals the presence of proteins and amino acids.

PriceTest for Carotenoid: Carr-price Reaction: 10 mL of extract was evaporated to dryness, and a few drops of a saturated solution of antimony trichloride in chloroform were added. A blue-green color eventually turned red, showing that carotenoids are present.

Test for carbohydrates: starch test: 1 mL of extract was dissolved in 5 mL of distilled water and filtered. The appearance of blue coloration showed that starch was present.

Test for reducing sugar: Benedict test: 0.5 mL extracts were dissolved individually in 5 mL of distilled water and filtered. Then 1 mL of Benedict solution was added and boiled for 1 minute. The appearance of a green, yellow, or red color signifies that reducing sugars are present.

Test for phytosterols: Salkowski test: 1 mL of extract was added to a few drops of concentrated sulfuric acid (shake well and allow to stand). The red color in the lower layer signifies the presence of phytosterol.

Test for cholesterol: acetic anhydride test: 0.5 mL of extract, 2 mL of acetic anhydride, and then 2 mL of concentrated sulfuric acid. A change of color from violet to blue or green shows that cholesterol is present.

Test for resin: Acetic anhydride test: 0.5 mL of extract was added to 2 mL of acetic anhydride and then 1 mL of sulfuric acid. The change from orange to yellow indicates that resins are present.

Test for carboxylic acid: effervescence test 1 mL of extract was added to a few drops of sodium carbonate powder. The appearance of effervescence signifies the presence of carboxylic acid.

3.5.3.1 Quantitative Phytochemical Determination

The determination of phenols, tannins, alkaloids, flavonoids, saponins, carotenoids, glycosides, saponins, and terpenoids was based on ultraviolet spectrometry (UV spectrophotometry) as per the protocol earlier laid down by [34][35][36][36][37] [38] [39], with modifications.

Determination of total Polyphenols

based The total polyphenol was determined based on the guidelines earlier laid down by [38] and [39], with modifications. The total phenolic compounds were calculated using the Folin-Ciocalteu reagent. In a nutshell, test tubes containing 100 mg of samples were filled with 10 mL of 50% methanol and divided into two portions of 5 mL each. To ensure thorough mixing, the samples were physically shaken for 30 to 40 minutes. The sample extracts were centrifuged for 10 minutes at 300 rpm and then kept at -20 ° C The extracts were decanted into test tubes, and 5 mL of distilled water was used to dilute 5.0 mL of the Folin-Ciocalteu reagent (2N). 0.1 mL of distilled water, 0.25 mL of diluted Folin-Ciocalteu reagent, and 1.25 mL of 20% sodium carbonate were used to dilute each sample in three equal parts. After the setups have stood for 35 minutes, the tubes are vortexed for another 15 minutes. The absorbance was measured at a wavelength of 725 nm. In order to create a standard solution, the following concentrations of garlic acids were made: 10, 50, 100, 250, 300, 350, 400, 450, and 500 mg/mL in methanol. The amount of phenol in the sample served as the basis for the creation of calibration curves. Gallic acid equivalents (mg GAE/g dry fruit weight) were used to translate the results into mg of dried extract.

Determination of total Tannins

Each powdered sample was weighed out to a total of five hundred milligrams (500 mg), and then it was placed into a 100-mL plastic bottle. In a mechanical shaker, 50 mL of distilled water was added, and it was agitated for an hour. After filtering, the mixture was produced to the proper consistency in a 50-mL conical flask. The filtrate was then pipetted out in 5 mL portions into a tube, where it was combined with 3 mL of 0.1 M FeCl₃ in 0.1 N hydrochloric acid and 0.008 M potassium ferrocyanide. Within 10 minutes, the absorbance at 530 nm was measured with a spectrophotometer[38]. The stock solution was prepared by

dissolving 100mg of tannic acid (Sigma) in 1 L of distilled water in a calibrated flask. Standard solutions were obtained by appropriate dilution of the stock solution with distilled water. 1.485g of 1910-phenanthroline monohydrate (Sigma) in distilled water and diluting 500 mL with the same. From this stock solution, a standard solution of 0, 1.5, 0.3, 0.6, 1.2, 1.5, 1.8, 2.1, and 2.4 mg/mL was prepared. The concentration curves were generated at 530 nm, and from these solutions, the concentration of tannins was determined.

Determination of total Flavonoids

A technique described by [39] was used to spectrophotometrically determine the total flavonoid. Test tubes were filled with 2.0 mL of the ethanol extract and received an equal amount (2 mL) of a 2% AlCl₃ solution. After one hour of incubation at room temperature, the solution's absorbance at 420 nm was measured. Rutin was dissolved in 100 mL of methanol as a stock solution, or at a concentration of 1 mg/mL, to create a standard curve. With distilled water, the stock solution was diluted to concentrations of 0, 1.5, 0.3, 0.6, 1.2, 1.5, 1.8, 2.1, and 2.4 mg/mL. Rutin equivalents (mg rutin equivalents per g) were then used to express the results. After that, the total flavonoid contents were determined. Rutin equivalents (mg rutin equivalents per g) were then used to express the results. Using the calibration curves that were produced, the following equation was used to determine the total flavonoid levels as rutin (mg/g).

Determination of total Alkaloids:

The protocol outlined in the preceding sentence by [40] was modified for the determination of alkaloid. A finely powdered portion of the plant material (100 mg) was then extracted continuously for 24 hours with methanol (Soxhlet). After filtering the extraction, methanol was vacuum-vaporized in a rotating evaporator at 45 °C until it was completely dry. Some of this residue was filtered after being partially dissolved in 2N HCl. It was then transferred to a separatory funnel; 1 mL of this solution was three times rinsed with 10 mL of chloroform. The pH was changed by adding 0.1 N NaOH to the mixture. The solution was supplemented with 5 mL of phosphate buffer and 5 mL of BCG. 5 mL of phosphate buffer and 5 mL of BCG were added to the solution. Shaking the mixture vigorously allowed the complex to form, which was then extracted using 1, 2, 3, and 4 mL of chloroform. The extract was gathered in a 10-mL flask and diluted with chloroform until volume was reached. At 470 nm, the complex's absorbance was measured. Atropine standard solution was properly measured into aliquots (0.4, 0.6, and 1.2 mL), and each was then transferred to separator funnels to create the stock solution. Bromocresol Green (BCG) solution and pH phosphate buffer were also added in amounts of 5 mL each, and the mixture was shaken with 1, 2, 3, and 4 chloroforms. At 417 nm, the absorbance was measured, and using calibration curves, the concentration of alkaloids was calculated.

Determination of total Terpenoid

The procedure previously described by [41] was modified to determine the total terpenoid content (TTC) of the extract of *Rhoicissus tridentata* (L.f.) Wild and R.B. Drumm. 100 mg of pulverized plant material were then continuously extracted with methanol for 24 hours (Soxhlet). The extraction was filtered, and

methanol was dried by vacuum-sealed rotary evaporation at a temperature of 45 ° C. 1 mL of the extracts were diluted with 2 mL of chloroform. After fully vortexing the sample mixture, it was left for three minutes. The mixture was then treated with 200 l of concentrated sulfuric acid (H₂SO₄) and incubated for 1.5–2 hours at room temperature in the dark. During incubation, a reddish-brown precipitate was created in the mixture. The supernatant was then carefully decanted without disturbing the precipitate. 3 mL of 100% methanol was added, and the mixture was vortexed vigorously until the precipitation had completely dissolved in methanol. A visible spectrometer (V-730 UV-Vis Spectrophotometer, Jasco, USA) was used to measure absorbance at 538 nm. The extracts' TTC was calculated as mg of linalool per gram of dry weight, or DW, of extract. The standard curve's equation was $y = 0.0036 \times 0.001$, with $R^2 = 0.9927$.

Determination of total Carotenoid

The study adopted the procedure previously described by[42] for quantification with modification. The plant materials (100mg) were ground and then extracted with methanol for 24 hours in continuous extraction (Soxhlet). The extraction was filtered, and methanol was evaporated in the rotary evaporator under vacuum at a temperature of 45 oC to dryness. Chemicals and solvents used in this assay were analytical grade and were acquired from POCH (Gliwice, Poland). To precipitate carotenoids, approximately 1.5 g of the sample was treated with Carrez I and II solutions. Afterwards, the extraction was carried out using acetone and petroleum ether. The absorbance value of the ether extract was measured at 450 nm. The standard solution was prepared by mixing 560µL of tool with lutein, -cryptoxanthin, lycopene, 250µL of carotene, and 1.00 mL of phytoene stock solution, then diluting to 5000µL with 50:50 ethanol and acetonitrile. Then, from this, stock solutions of 1.5, 0.3, 0.6, 1.2, 1.5, 1.8, 2.1, and 2.4 mg/mL were prepared. It was determined from calibration curves obtained from the standards.

Determination of total Glycosides

The procedure previously described by[43] for quantification was adopted with modifications in the standard solution of luteolin-7-glycoside. The plant materials (100mg) were ground and then extracted with methanol for 24 hours in continuous extraction (Soxhlet). The extraction was filtered, and methanol was evaporated in the rotary evaporator under a vacuum at a temperature of 45 ° C to dryness. 10 mL of freshly made Baljet's reagent (95 mL of 1% picric acid and 5 mL of 10% NaOH) was combined with a 10% extract of each generation and a total extract of seeds to determine the presence of cardiac glycosides. In order to test the absorbance at 495 nm, the mixture was diluted with 20 mL of distilled water after an hour. A 1.0 mL standard solution of luteolin-7-glycoside was dissolved in a 25- mL flask with 2 mL of a 2% aluminum chloride solution to create the luteolin-7-glycoside. Different quantities of 1.5, 0.3, 0.6, 1.2, 1.5, 1.8, 2.1, and 2.4 mg/ mL were generated in this stock solution, and absorbance was measured at 495 nm to quantify these amounts of glycosides in the sample.

Determination of total Saponin

The method for quantification originally reported by[44], was used, although with changes in the standard solution of anisaldehyde reagent. The plant material (100 mg) was crushed before being continuously

extracted with methanol for 24 hours (Soxhlet). The extraction was filtered, and methanol was dried by rotary evaporation at a temperature of 45 °C while being vacuum-sealed. 1.11 g of the natural sample (or 1.11 g of the corresponding dry weight for processed samples) was mixed with 10 mL of 50% ethanol to extract the saponins, which were then allowed to macerate for 72 hours at room temperature. The extracts were then filtered into 10-milliliter volumetric flasks and finished with 50% ethanol. 7 mL of Lieberman-Burchard reagent (16.7% of acetic anhydride in concentrated sulfuric acid) was mixed with 2 mL of the diluted extract (1:25 dilution), or standard, to complete the analysis. The solution was vortexed and scanned at 528 nm after standing for 30 minutes at room temperature. The dry material was quantified using a standard saponin curve (50–350 g/mL), and the findings were reported as mg/g.

Statistical Analysis

All the data was analyzed by GraphPad Prism® version 9.0.0. The Shapiro-Wilk test revealed that all the photochemical constituents were not normally distributed ($p \geq 0.05$) except saponin in the aqueous extract ($p \leq 0.0194$). The Kruskal-Wallis test was used to test for significant medium concentration differences among the same compound and different groups of compounds, and finally, the post-hoc test (Mann-Whitney tests) was used to locate the medium significant difference. Significant differences for saponins were determined by an independent student t-test. Data were expressed as mean $\pm$ standard deviation and summarized in tables in the following proceeding. Graphs were generated by the GraphPad prism.

RESULT & DISCUSSIONS

Results

The current study evaluated the phytochemical makeup of root tubers' of *Rhoicissus tridentata* (L.f.) Wild and R.B. Drumm that were harvested in the dry months of February and March from the slopes of Mount Elgon (Sironko and Bulambuli districts). At the biology laboratory at the Islamic University of Uganda, the plant materials were right away washed with clean water, cut into little pieces, and air-dried. The dried materials were taken to the chemical laboratory at Makerere University, where a qualitative examination was completed, and to the natural chemotherapeutic laboratories, where a spectrophotometric method for quantification was used.

Table 1: Qualitative Phytochemical analysis of methanol and aqueous extracts of *Rhoicissus tridentata* (L.f) Wild root tubers.

SN.	Phytochemical Compounds	Test procedure	Expected color	Methanol extracts	Water extracts
1	Phenol	To 2mL of plant extracts, were added a few drops of 5 % ferric chloride solution	The appearance of dark green/blue-black color, indicates the presence of phenol.	+	+
2	Tannins	To 1 mL of the extract, was added 3 mL of the distilled water and then 10% ferric chloride solution.	The presence of tannins was detected by dark-green color	+	+
3	Phlobatanins	To 2 mL of the extracts, were added 2 mL of 1% HCl and boiled.	Appearance of red precipitate shows the presence of phlobatanins	+	+
4	Alkaloids	To 3mL of the extracts were added a few drops of iodine and the mixture was boiled	A blue color that disappears on boiling and reappears on cooling showed the presence of alkaloids	+	+
5	Saponins	0.5 g of extract was diluted with 2 mL of distilled water and then shaken well in a test tube for 15 min,	The appearance of persistent foam for 10 minutes reveals the presence of saponins.	+	+
6	Flavonoids	1 mL extract was added to 2 mL of 2 % sodium hydroxide solution, followed by 2 M HCl	An intense yellow color that becomes colorless with the addition of dilute acid will signifies the presence of flavonoid.	+	+
7	Anthraquinone	10 mL of 10 % ammonia solution, was added to a few of the filtrates (shake vigorously for 30 seconds).	Pink, violet, or red-colored solution indicates showed the presence of anthraquinone	+	+
8	Anthocyanins	2mL extract, was added 2mL M HCl (+ few drops of ammonia).	The appearance of the pink-red solution turns blue-violet after the addition of ammonia, showed that anthocyanins are present.	-	-
9	Terpenoids	0.5g of the extracts was added to 2 mL of	The appearance of reddish-brown color at	+	+

		chloroform and evaporated to dryness. Then, 3 mL of concentrated sulphuric acid added.	the interphase reveals the presence of terpenoids.		
10	Triterpenoids	2 mL extract, was added few drops of concentrated sulphuric acid (shaken well and allowed to stand)	Appearance of a golden yellow layer (at the bottom) signifies the presence of triterpenoid.	+	+
11	Steroids and sterols	5 mg of extract was dissolved in 2 mL of chloroform and an equal volume of concentrated sulphuric acid was added along the sides of the test tube	Once the upper layer turns red and the lower layer turns yellow with green fluorescence, indicate the presence of the steroids and sterols compound, in the extract.	+	-
12	Quinones	1 mL of the extract was added a few drops of concentrated HCl.	The appearance of green color showed that quinone is present.	-	-
13	Fixed oils and fats	A little quantity of the extract was placed between the filter papers.	Observed oil stains on the paper, showed that fixed oils are present.	-	-
14	Volatile oils	Extract is applied on the filter paper:	A transparent appearance (oils and resins) will show that volatile oils are present.	-	-
15	Glycosides	2mL hydrolysate, was added 3 mL of chloroform, and shaken well, chloroform Layer was separated, and then 10 % ammonia was added	The appearance of a pink-colored solution indicates the presence of a glycosides	+	+
16	Cardiac glycosides	1 mL extract add 1.5 mL of glacial acetic acid and 1 drop a few ferric chloride solutions and finally concentrated sulphuric acid (alongside the test tube).	A blue-colored solution in (the acetic acid layer) signifies that cardiac glycosides are present.	-	-
17	Coumarins	2 mL of the plant extract, was added 10 % of NaOH and then chloroform.	The appearance of yellow color shows the presence of coumarins	+	+
18	Proteins and amino acids	2 mL extract, was added a few drops of millon's reagent.	A white precipitate formed, indicate the presence of protein and amino acids.	+	+

19	Carbohydrates	1 mL extract, was added 5 mL of 5 % KOH.	Acinary coloration shows that starch is present.	+	+
20	Reducing sugar	0.5 mL extract, add 0.5 mL benedict reagent and boil for 1 minute.	Green/yellow/ red coloration indicates that reducing sugars are present	+	+
21	Phytosterols	1 mL extract was added a few drops of concentrated sulphuric acid (shaken well and allowed to stand).	Appearance of the red color in the lower layer signifies the presence of phytosterol.	+	+
22	Cholesterols	0.5mL extracts were added 2 mL of acetic anhydride, followed by 2 mL of concentrated. Sulphuric acid.	A change of color from violet to blue/green shows that cholesterols are present.	-	-
23	Resin	0.5mL extract was added 2 mL of acetic anhydride, followed by mL of sulphuric acid.	The change of orange to yellow, indicates that resins are present.	+	+
24	Carboxylic acid	1 mL of extract, was added a few drops of sodium carbonate powder.	The appearance of effervescences, indicates the presence of carboxylic acid.	-	-

+ (+) Present and (-) Not detected

According to the current study's findings, *Rhoicissus tridentata's* root tubers were highly embodied with a variety of phytochemical components in both methanol and aqueous extracts. In terms of steroids and sterols, only phytochemical content was found in methanol but not in aqueous extracts. However, some phytochemical substances, including anthocyanins, quinones, fixed fats, volatile oils, cholesterol, and carboxylic acid, were not at all found in any extract.

Table 2: Concentration of Phytochemical Compounds in methanol and aqueous extracts of *Rhoicissus tridentata* (l.f) Wild root tuber

Phytochemical Composition	Medium± SD		Standard Error		95 % Confidence interval (CI)		P – Value
	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	
Alkaloids	117.3 ± 2.67	117.3 ±5.244	1.090	1.090	114.1 to 119.7	114.1 to 119.7	p ≥ 0.9999
Saponins	172.6 ±3.732	18.12 ±3.732	0.07661	0.1259	169.9 to 177.7	17.21 to 18.75	p≤ 0.0022*
Carotenoids	29.92 ±1.066	33.28 ±2.336	0.4354	0.9535	27.81 to 30.05	30.26 to 35.16	p≤ 0.0022
Polyphenols	129.6 ±0.587	343.0 ±1.132	0.2398	0.4620	129.1 to 130.4	342.1 to 344.5	p≤ 0.0022
Flavonoids	11.01 ±0.188	27.88 ±0.7328	0.3616	0.1605	10.81 to 11.21	27.45 to 28.10	p≤ 0.0022
Terpenoids	29.54 ±1.597	33.28 ±0.8858	0.6521	0.3616	26.41 to 29.76	32.55 to 34.41	p≤ 0.0022
Tannins	49.45 ±0.407	217.5 ±0.393	0.1605	0.1660	49.13 to 49.98	217.4 to 218.4	p≤ 0.0022
Glycosides	124.8 ±5.244	124.8 ±4.668	2.141	2.141	118.6 to 129.6	118.6 to 129.6	p≥ 0.9999

(*)- Independent student t-test and rest without * Mann-Whitney test

The current study showed a substantial variation in several phytochemical components for *Rhoicissus tridentata* (L.f.) Wild & R. B. Drumm extracts in both methanol and aqueous extracts. There were a lot more alkaloids, polyphenols, tannins, and glycosides in methanol extracts than in water extracts (p≤ 0.0022). Saponin was found to be significantly (p≤ 0.0022) more soluble in water than methanol.

Discussions

In the present study, the phytochemical components of *Rhoicissus tridentata* (L.f.) root tubers collected from Mount Elgon's slopes between February and February 2023, a dry season, were analyzed. The plant materials were cleaned, chopped into small pieces, and air-dried at Islamic University in Uganda (IUIU) prior to their transportation to the Chemistry at Makerere University and natural chemotherapeutics laboratories, where qualitative and quantitative research were respectively conducted. GraphPad Prism® version 9.0.0 was used to analyze the phytochemical contents. The Shapiro-Wilk test revealed that the saponin concentrations in the aqueous extract were uniformly distributed (p≤0.0194). The remaining phytochemical components in both the aqueous and methanol extracts were not normally distributed (p≥0.05). The Kruskal-Wallis test showed a significant difference among the different phytochemical compounds in both extracts. The mean difference between the same phytochemical components of the two extracts was examined using Mann-Whitney's test. A student's independent t-test was used to examine the mean difference in saponins between aqueous and methanol extracts.

A rich amount of plant active metabolites known to have anticancer effects was found in the root tuber of *Rhoicissus tridentata* (L.f.) Wild & R.B. Drumm. (Table 1, Figure 3 & Figure 4). The presence of these phytochemicals has also been noted in earlier studies conducted in other parts of the world, though with some variations due to their sensitivity to a number of variables, such as extraction techniques, harvesting season, species, habitat conditions, storage time, and conditions. Several species in the genus *Rhoicissus* that were taken from various locations in South Africa were found to have essential oil and phenolic compounds, among other significant and crucial chemical compounds[9]. Their conclusions were different from those of the present investigation in terms of the essential oil[9]. This is explained by the diversity of *Rhoicissus* species used and the various ecological conditions. The plant species included in previous investigations were gathered during both the dry and wet seasons from a variety of habitat circumstances, in contrast to the current study, which only collected plant samples during the dry season [10]. The variation in phytochemical content is influenced by all of these elements, both qualitatively and quantitatively. Catechins, epicatechins, gallic acid, and epigallo-catechin-gallate were found in *Rhoicissus tridentata* (wild) collected from several South African locales but not explicitly examined in the present study[45]. This variance may be explained by the particularity of the illness under investigation in the context of the current study. For instance, whereas the prior work by [45] focused on antimicrobial phytochemicals as a significant factor, the current study is interested in phytochemical content with anticancer activities. The phytochemical constituents of *Rhoicissus tridentata* (L.f.) Wild & R.B. Drumm., taken from the Elgon sub-region, shared the same phytochemical contents as those of *Cayratia pedata* Vara. glabra (Vitaceae) [46]. However, unlike the current study, volatile oils were detected in acetone extract and ethanol; solvents were not employed in this regard. Due to changes in the ecological circumstances where the plants were taken (the species in issue) and solvents in prior research, variations in extraction methods, alkaloids, and saponins were reported to be absent[46]. Methanol was used in our study instead of ethanol as a better solvent[47]. The *Rhoicissus tridentata* (L.f.) Wild and R.B. Drumm root tubers used in this study were obtained from the Elgon sub-region, which has different ecological zones from those of India where their plant samples were obtained, which is responsible for the phytochemical variations between the two studies[47].

The present study found that the root tubers of *Rhoicissus tridentata* extract exhibit varied concentrations in both methanol and aqueous extracts. Polyphenols, tannins, glycosides, and terpenoids were significantly ($p \leq 0.0022$) higher in methanol extract with respect to aqueous. On the other hand, aqueous substantially ($p \leq 0.0022$) served as a better extract for saponins as compared to methanol. Previous studies on other species of Vitaceae found lower levels of total phenolics (54.1 ± 1.8) and tannins (10.5 ± 3.2) compared to the current study (phenol, 129.74 ± 0.587 and tannins, 49.5 ± 0.407). (Figure 2 and Table 2). The distinctions can be attributable to the fact that different species' phytochemical compositions have been observed, even among members of the same family and plants cultivated in similar habitats[14]. With phenol at 97.58 ± 0.09 and flavonoids at 97.47 ± 0.12 , the levels of total phenol in our sample were higher (129.74 ± 0.587) but lower (11.01 ± 0.188) than in the report mentioned by[48]. The species in question and the plant parts used are to blame for this. The former experiment used leaves from *Vitis venifera* (Vitaceae), in contrast to the current analysis, which only utilized the root tubers of *Rhoicissus tridentata*.

Conclusions and Recommendations

The research found that various anticancer bioactive chemicals were significantly concentrated in the root tubers of *Rhoicissus tridentata* (L.f.) Wild and R.B. Drumm, and that these concentrations were typically higher than those found in other parts of the world. Methanol worked better as a solvent for polyphenols, tannins, alkaloids, and glycosides than it did for saponins in an aqueous solution. These extracts need to be subjected to in vitro and in vivo studies, as well as the isolation and characterization of the most bioactive chemicals, in order to aid the pharmaceutical industry in the development of novel and more effective anticancer drugs.

Declarations

Statement on the relevant institutional, national, and international guidelines and legislation followed in the research and collection Plant Materials

This study is based on the Nagoya protocol for the collection of plant material, as earlier described by [49] & [50], with some modifications. All the work was conducted under the stipulations of the Nagoya protocol on access to genetic resources and fair and equitable sharing of benefits. arising from their utilization of the Convention on Biological Diversity. The right to use and authorship of any traditional knowledge of all participants is maintained, and any use of this information, other than scientific publication, does require the prior consent of traditional owners as well as consensus on access to benefits resulting from subsequent use.

Ethical approval and consent to collect plant materials

The School of Biomedical Sciences Research Ethics Committee at Makerere University approved this study (SBS-2023-320). Local officials from Sironko and Bulambuli, including the LC1 chairpersons, granted permission to visit these settlements and collect plant materials. The researchers received consent prior to the collection of materials from herbalists for laboratory investigations.

Consent for Publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study was included in this article.

Competing Interests

The authors declare that there is no conflict of interest.

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Authors' Contribution

AK, JNK, HM, GSB, AMD, and MO contributed to the conceptualization of the study and revision of the manuscript. AK, JES, SOA, HMK, ML, and AW gathered and analyzed the data and wrote the draft manuscript. JNK, HM, GSB, HW, AL, and NN were supervisors and senior advisors in the study. They reviewed, read, and approved the final manuscript. All the authors read and approved the final manuscript.

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Figures

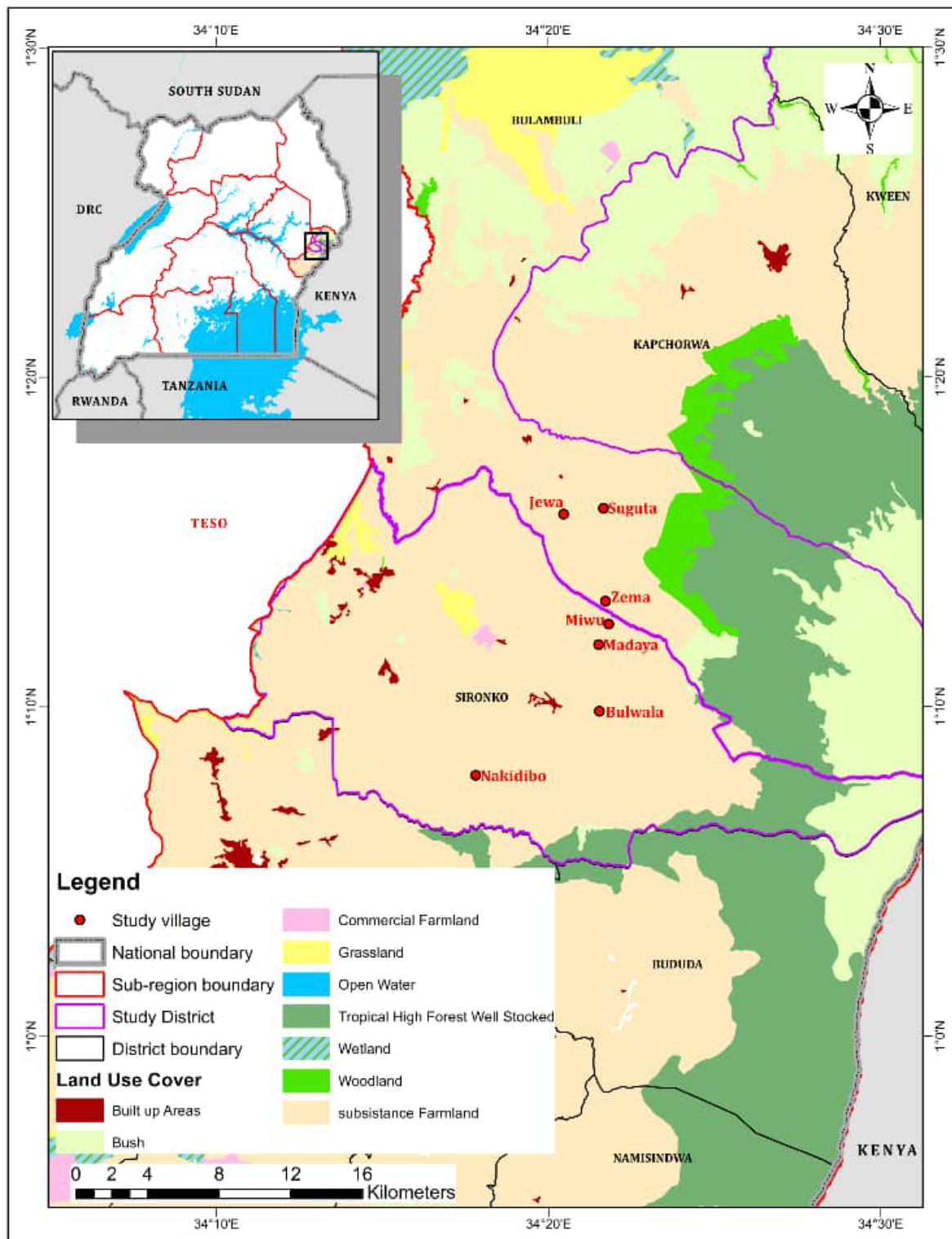


Figure 1

Map of Elgon Sub-region with Location of Sironko and Bulambuli districts

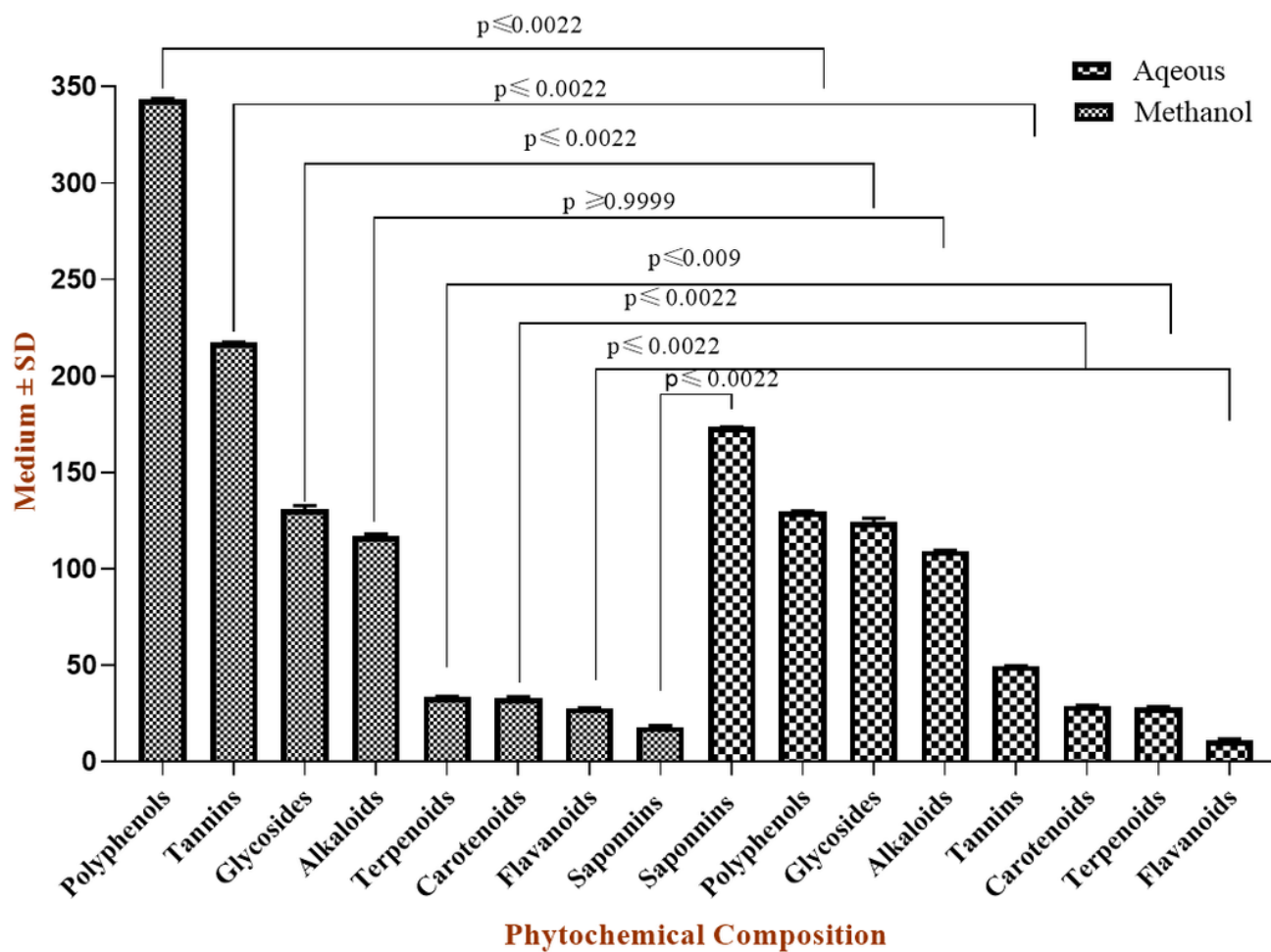


Figure 2

Concentration of Phytochemical Compounds in Methanol and Aqueous Extracts of *Rhoicissus tridentata* (L.f) Wild

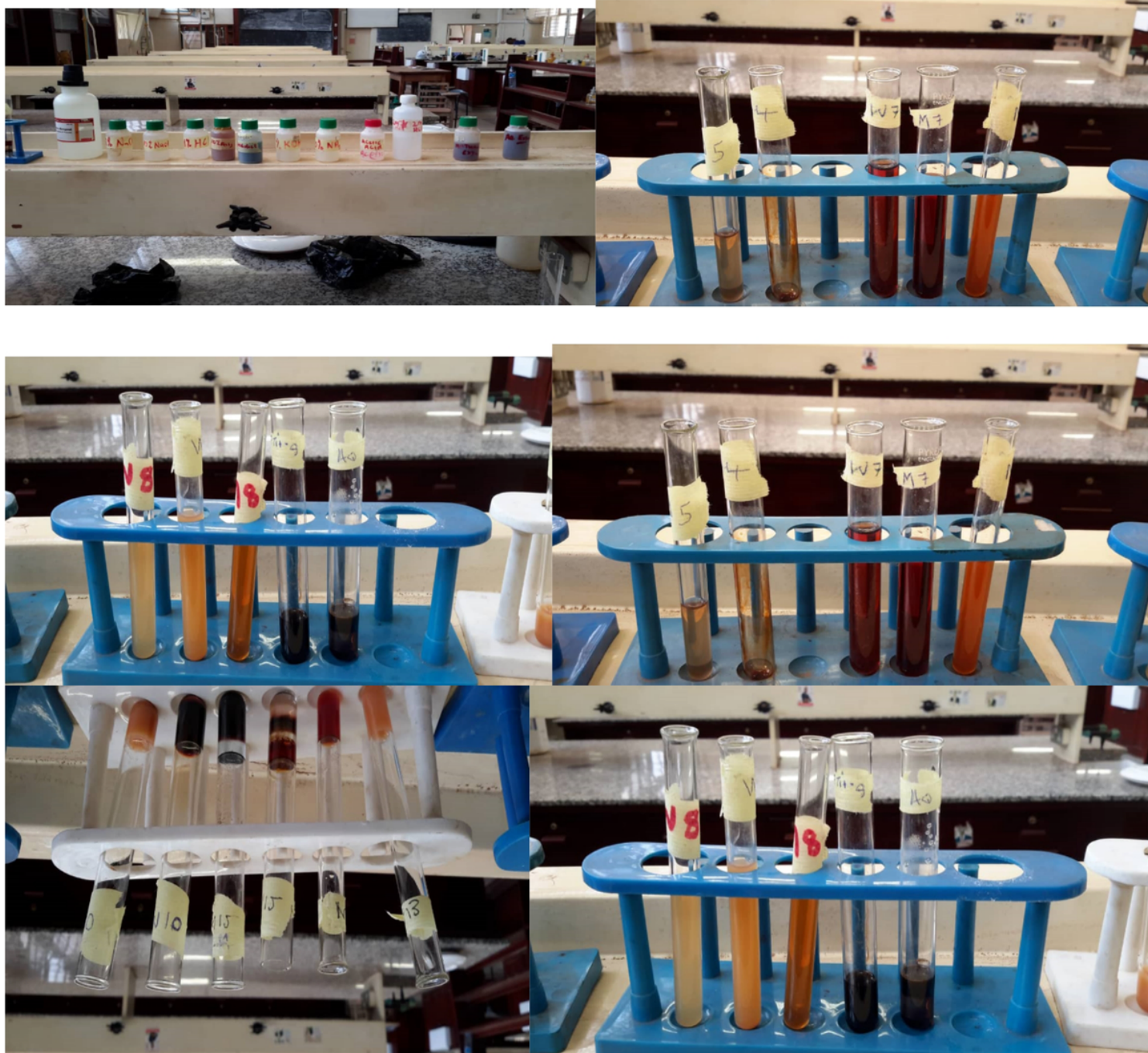


Figure 3

Qualitative experimental tests conducted for qualitative phytochemical analysis in the Chemistry Laboratory at Makerere University, Kampala

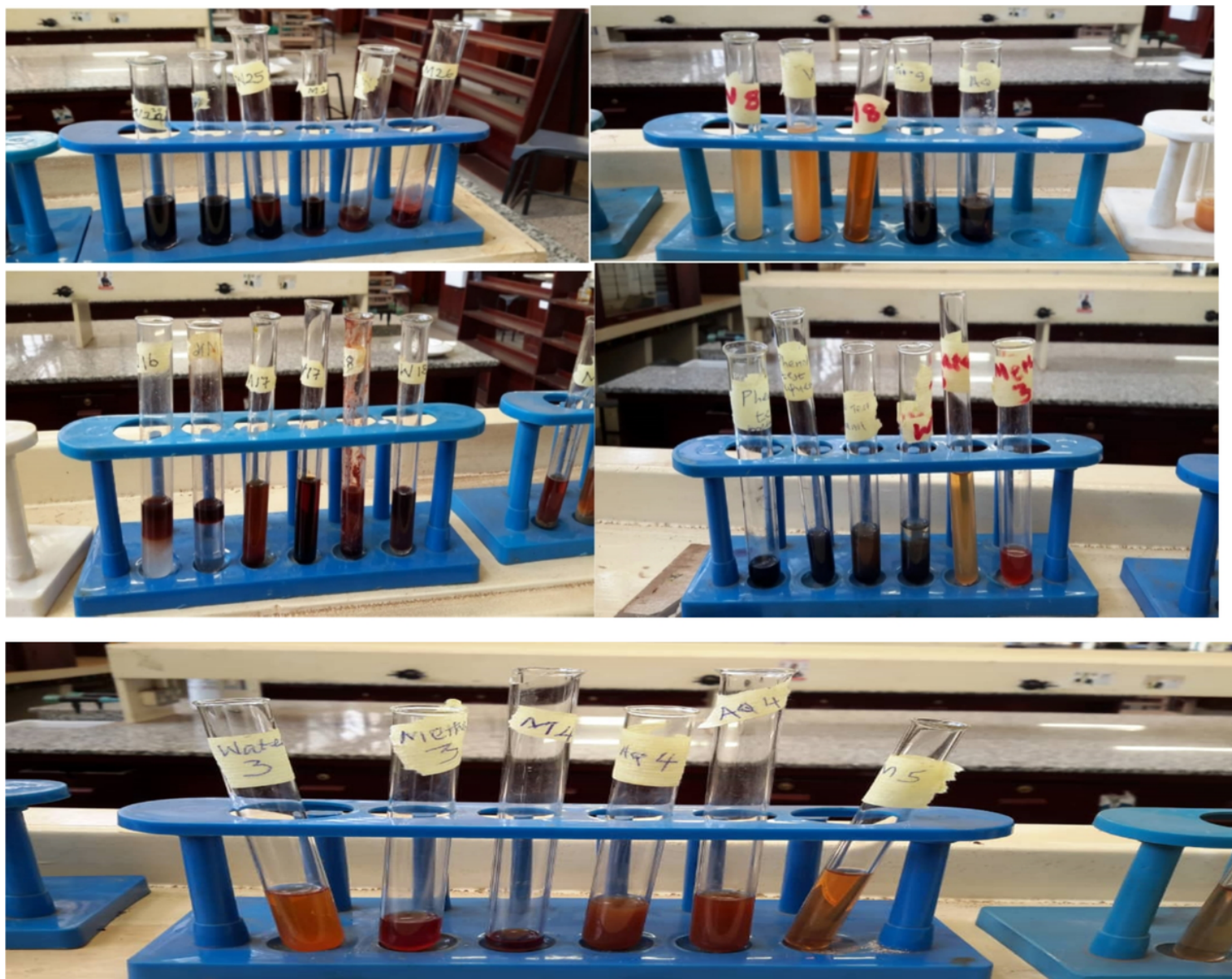


Figure 4

Qualitative experimental tests conducted for qualitative phytochemical analysis in the Chemistry Laboratory at Makerere University, Kampala