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Preliminary Phytochemical Screening and Quantitative Analysis of Methanol Leaf Extract of *Erlangea tomentosa* (Oliv. & Hiern) S.Moore (Asteraceae)

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Abstract

Various medications are being developed using natural products, particularly those resulting from medicinal plants. So, the screening and quantity analysis of phytochemicals in the methanol leaf extract of *Erlangea tomentosa* were looked at to find out what chemicals in the plant were responsible for its biological activity. Ten different phytochemicals, such as alkaloids, glycosides, cardiac glycosides, flavonoids, saponins, coumarins, steroids, terpenoids, phenols, and tannins, were found by the analysis. Glycosides and cardiac glycosides were below the limits, and neither phlobatannins nor anthraquinones were found. Quantitative phytochemical analysis showed that there were 3.38 %(w/w) of total alkaloids, 2.19%(w/w) of total tannins, 1.81%(w/w) of total flavonoids, and 0.31%(w/w) of total saponins.

Keywords: *Erlangea tomentosa*, phytochemistry, quantitative analysis, methanol leaf extract

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1 Introduction

Medicinal plants continue to be an interesting source of natural products for treating various health conditions. It is estimated that more than 150,000 plant species have been studied. Many of which contain valuable therapeutic agents, and the applications of novel compounds from plants for pharmaceutical purposes have been gradually increasing in recent years [1].

In an adaptation against attacking pathogen and environmental stress, plants produce several substances that exert biological activities. These small organic molecules come from secondary metabolism and have several medical properties. Naturally, phytochemicals are secondary metabolites, biosynthesized from primary metabolites, eliciting pharmacological or toxicological effects in man and animals [2]. Alkaloids, flavonoids, saponins, terpenes, tannins, coumarins, glycosides, phenolics, anthraquinones, essential oils, and steroids are examples of phytochemicals [3]. *Erlangea tomentosa* (Oliv. &Hiern) S.Moore (Asteraceae) is a woody erect herb that can reach a height of 50 cm. In Runyankore and Kirundi, it is known respectively as *Ekyoganyanja,* and *Umubebe*. In open patches of woodland, shrubby growth from 7.5cm to 25cm high, typically completely exposed to sunlight, is normal. Orthodox herbal medicine practitioners commonly use the leaves of this plant to make herbal remedies for a variety of ailments such as stomachaches, syphilis, fever, as well as miscarriage [4]. *E. tomentosa* has been shown as promising antioxidant potential in Asiimwe's thesis in Uganda [5]. Recently, a 2020 study attributed *E. tomentosa* to possess analgesic and anti-inflammatory properties in mice and rats [4].

So, the leaves of the plant are a rich source of phytoconstituents that can be efficiently used as drugs which are believed to be responsible for a range of protective and curative health benefits including antioxidative, anti-inflammatory, antitumor, and antimicrobial activities [5]. The biological activities of *E. tomentosa* are becoming well-documented. Despite its long history of use and the known biological properties of this medicinal plant, there have been no sufficient reports of its secondary metabolites in various extract solvents and in the amounts associated with them. The goal of this study was then to do a wide phytochemical screening, including a quantitative analysis of the major secondary metabolites in the crude methanol leaf extract of *E. tomentosa* (MLEET) in order to find out what chemicals are responsible for its different biological properties.

2 Materials and Methods

2.1 Collection, identification, and extraction of the plant material

The leaves of *E. tomentosa* were hand-picked from Bwegiragye village, in Bushenyi District, Western Uganda. The plant was identified by a plant taxonomist from Mbarara University of Science and Technology (MUST). A voucher specimen with the number B50891 was deposited in the herbarium of the Department of Botany for future reference. Plant extracts were prepared according to established approaches [6] [7]. To summarize, newly collected plant leaves were washed 2-3 times with tap water to eliminate adherent particles before being dried in the shade until a consistent mass is achieved. With the aid of a mortar and pestle, the dried material was ground into a fine powder. The powdered materials were packed in closed dry khaki paper bags at room temperature, out of direct sunlight.

Using an Erlenmeyer flask, we macerated this coarsely powdered plant (1g) in 70 percent methanol (10ml) for 3 days at room temperature to obtain the hydro-alcoholic crude extract. Filter paper was used to remove the filtrate from the marc after 72 hours (Whatman No.1) [6]. The alcohol was then enabled to vaporize from the filtrate using a rotary evaporator under reduced pressure and more concentrated in a water bath at 55°C. The condensed extract was maintained at 4°C till the experiment was over [7].

2.2 Phytochemical screening

This was done using chemicals of analytical grade following methods described by Trease and Evans [8] and Lamara et al.[2]. All analyses were done at the Pharmaceutical Analysis Laboratory, Faculty of Medicine and Pharmacy, Mbarara University of Science and Technology (MUST), Uganda.

2.2.1 Extract solution Preparation

To make a test solution for chemical testing, we had to weigh 2.5 g of plant extract and mix it with 25 mL of deionized water in a marked container [2].

2.2.2 Identification of alkaloids

The Dragendorff's reagent (DR) was utilized in the performance of the test. A 1% solution of hydrochloric acid and a few droplets of DR were mixed into 2 mL of sample. Alkaloids were produced by the development of an orange-scarlet sediment [8].

2.2.3 Identification of saponins (The froth formation test)

After rapidly shaking the mixture of 2 mL of sample combined with 2 mL of deionized water and then left to stand for half an hour, the soapy foam that lasted for half an hour shows that saponins were present [8].

2.2.4 Identification of tannins and phenols (Ferric Chloride test)

A mixture of 2mL of sample and 2mL of 5% $FeCl_3$ was prepared. A formation of intense-blue dark coloration reveals that phenols and tannins were present [9].

2.2.5 Identification of flavonoids

A mixture of 2mL each of NaOH solution (2N) and extract was prepared. Flavonoids could be identified by their characteristic yellow hue, which appears when they are present [2].

2.2.6 Identification of terpenoids and steroids (Salkowski approach)

2 mL each of $CHCl_3$ and extract were prepared, and thereafter, slowly added 2 mL of concentrated sulfuric acid that creates a thin film on the surface. At the point where two layers met, terpenoids showed up as a red-orange ring. Steroids, on the other hand, were found by the change in color of the top layer, which went from yellow to blue or green [8].

2.2.7 Identification of coumarins

1mL of the sample was mixed with 1mL of 10% NaOH solution. Coumarins could be detected by the yellow coloring they produce [9].

2.2.8 Identification of glycosides

The extract (2 mL) was mixed with 3 mL of $CHCl_3$ and a 10% NH_4OH solution. The presence of glycosides is specified by the development of a pink tint [9].

2.2.9 Identification of phlobatannins

A 2% hydrochloric acid solution was heated, containing 2mL of sample. Phlobatannins' presence would be confirmed wherever a scarlet precipitate is developed [10].

2.2.10 Identification of cardiac glycosides (Keller-Killiani test)

A 2 mL of 100% CH_3COOH with one droplet of $FeCl_3$ solution was mixed with 2 mL of sample. This was toned down by using 1mL of concentrated H_2SO_4. The existence of deoxysugar, which is a hallmark of cardenolides, was revealed by the appearance of a chocolate loop at the interface. In the acetic acid film, a greenish loop may emerge slightly above the chocolate loop and eventually expand across this coat, while a purple loop might emerge underneath the chocolate loop [8].

2.2.11 Identification of anthraquinones

When a few drops of a 10% NH_4OH solution were put into 1 mL of sample, a pink sediment formed. This showed that anthraquinones were present [10].

2.3 Quantitative analysis of methanol leaf extract of *E. tomentosa*

According to Lamara and colleagues (2020) as well as Madhu and collaborators (2016), analytical UV/techniques were utilized to quantitatively express phytochemical compounds of *E. tomentosa* such as alkaloids, flavonoids, tannins, and saponins [2] [11].

2.3.1 Analysis of total alkaloids content

To express the quantity of total alkaloids, a solution of quinine sulphate was utilized as a reference solution (0.1 g in 100 mL of deionized water). Pipetted 0.2, 0.4, 0.6, 0.8, as well as 1.0 mL of the reference solution into different test tubes and completed with pure water to volume of 10 mL. 3mL of Dragendorff's solution (DR) were added in each test solution, accompanied by 3mL of thiourea mixture (made by solvating 3.0 g in 100 mL deionized water). The absorbance readings at 435 nm of the test solutions generated were evaluated versus uncolored reagent blanks utilizing a spectrophotometer. A standardization curve of absorbance against concentration reference

solution was conspired and appeared to be linear (figure 2).

Each of 10mL of the three crude sample solutions (made by solvating 0.1 g of plant extract in 100 mL of the beaker) were drawn and transferred into different labeled test tubes. 3mL of DR, and 3mL of thiourea mixture, were added into each of the test tubes. At 435 nm, the absorbance was expressed to deionized water, which served as a blank. In order to calculate the total alkaloids' amount in mg per mL, the absorbance equivalent was plugged into the standardized curve's straight equation. DR was made by combining 0.8 g bismuth nitrate.$5H_2O$ in 40 mL deionized water and 10 mL of 100% acetic acid accompanied by a mixture of 8.0 g of potassium iodide in 20 mL pure water [2].

2.3.2 Analysis of total flavonoids content

To express the quantity of total flavonoids, a solution of quercetin was utilized as a reference solution (0.1 g in 100 mL of deionized water). Pipetted 0.2, 0.4, 0.6, 0.8, as well as 1.0 mL of the reference solution into different test tubes and completed with pure water to a volume of 10 mL. 3mL of DR were added in each test solution, accompanied by 3mL of thiourea mixture (made by solvating 3.0 g in 100 mL deionized water).

The absorbance readings at 510 nm of the test solutions generated were examined versus uncolored reagent blanks utilizing a spectrophotometer. A standardization curve of absorbance against concentration reference solution was conspired and appeared to be linear (figure 3).

Each of 10mL of the three crude sample solutions (made by solvating 0.1 g of plant extract in 100 mL of the beaker) were drawn and transferred into different labeled test tubes. 3 mL of DR, as well as 3mL of thiourea mixture, were added into each of the test tubes. At 510 nm, the absorbance was expressed versus deionized water, which served as a blank. In order to calculate the total flavonoids' quantity in mg/mL, the absorbance equivalent was plugged into the standardized curve's straight equation [12].

2.3.3 Analysis of total tannins content

To express the quantity of total tannins, a solution of tannic acid was utilized as a reference solution (0.1 g in 100 mL of deionized water). Pipetted 0.2, 0.4, 0.6, 0.8, as well as 1.0 mL of the reference solution into different test tubes and completed with pure water to a volume of 10 mL. 3 mL of iron (III) chloride (made by solvating 5.0 g in 100 mL deionized water) were put in each test solution. The absorbance readings at 550 nm of the test solutions generated were evaluated versus uncolored reagent blanks utilizing a UV-Visible spectrophotometer. A standardization curve of absorbance against concentration reference solution was conspired and appeared to be linear (figure 4).

Each of 10mL of the three crude sample solutions (made by solvating 0.1 g of plant extract in 100 mL of the beaker) were drawn and transferred into different labeled test tubes. 3mL of iron (III) chloride were put into each of the test tubes. At 550 nm, the absorbance was expressed to deionized water, which served as a blank. In order to calculate the total tannins' quantity in mg/mL, the absorbance equivalent was plugged into the standardized curve's straight equation [2].

2.3.4 Analysis of total saponins content

To express the quantity of total saponins, a solution of diosgenin was used as a reference solution (0.1 g in 100 mL of 80% of ethanol). Pipetted 0.2, 0.4, 0.6, 0.8, and 1.0 mL of the reference solution into different test tubes and completed with 80% of ethanol to a volume of 10 mL. 1mL of reagent A (made by solvating 800 mg of vanillin in 10mL of 99.5% of ethanol) was added in each test solution, accompanied by 1mL of reagent B (made by mixing 28mL of distilled water in 72mL of concentrated sulfuric acid 95%).

The absorbance readings at 544nm of the test solutions generated were evaluated versus uncolored reagent blanks (10mL of distilled water added 1mL of reagent A and 1mL of reagent B) using a UV-visible spectrophotometer. A standardization curve of absorbance against concentration reference solution was conspired and appeared to be linear (figure 5). Each of 10mL of the three crude sample solutions (0.1 g in 100 mL of 80% of ethanol) was drawn and transferred into different labeled test tubes. 1mL of reagent A was added in each test solution, accompanied by

1mL of reagent B. At 544 nm, the absorbance was expressed to10mL of distilled water added 1mL of reagent A and 1mL of reagent B, which served as a blank. To calculate the total saponins' quantity in mg/mL, the absorbance equivalent was plugged into the standardized curve's straight equation [13].

2.4 Data presentation

Statistical Package for the Social Sciences (SPSS) software was used to figure out the mean and standard deviation (SD) of the statistical data from the three replicate analyses. This was then interpreted as g per 100 g of plant extract.

3 Results and Discussion

The phytochemical screening of the methanol extract of the leaves of *E. tomentosa* revealed the existence of alkaloids, glycosides, cardiac glycosides, flavonoids, saponins, coumarins, steroids, terpenoids, phenols, and tannins (Table 1). The extract contained a high concentration of phytochemicals such as alkaloids, tannins, flavonoids, phenols, and coumarins. However, some other chemicals were moderately present, like saponins, terpenoids, and steroids. Additionally, glycosides and cardiac glycosides had weak-intensity reactions, while phlobatannins as well as anthraquinones were not detected. Flavonoids and alkaloids have a variety of therapeutic characteristics, including astringent, antioxidant, as well as analgesic properties [14]. Herbalists have used plant extracts for centuries to treat bacteria-related health issues [1]. Anti-inflammatory, antibacterial, and analgesic properties are all possible uses for *E. tomentosa.* This is attributed to the presence of flavonoids, saponins, steroids, alkaloids, and glycosides in their compositions [4].

Alkaloids are used to make drugs like caffeine, opiates, nicotine, ephedrine, and cocaine, so they are in more than half of the common psychoactive and social medicines that people take [1]. Flavonoids, which are a type of antioxidant phytochemical, are some of nature's most powerful compounds because they fight cancer and stop tumors from getting worse [15]. Several flavonoids have been studied for their anti-inflammatory properties, including apigenin, chrysin, diosmetin, quercetin, and kaempferol. When it comes to reducing the size of edema, quercetin and phenylbutazone are almost the same in how well they act for both short-term and long-term conditions [16].

Table1. Phytochemical components of the *E. tomentosa* plant extract

Phytochemical Constituent	MLEET	Phytochemical Constituent	MLEET
Alkaloids (Dragendorff's test)	**+++**	Phlobatannins test	-
Flavonoids (Ammonia test)	**+++**	Coumarins test	**+++**
Tannins and phenols test (Ferric Chloride test)	**+++**	Terpenoids and steroids (Salkowski method)	**++**
Saponins (froth formation test)	**++**	Cardiac glycosides (Keller-Killiani test)	**+**
Glycosides test	**+**	Anthraquinones test	-

Key- Heavily present: **+++**; moderate present: **++;** present: **+**; absent: **–** MLEET: Methanol leaf extract of Erlangea tomentosa

Table 2. Quantitative phytochemical analysis of *E. tomentosa*

Constituents	Mean values %(w/w)	95% CI for the mean
Total alkaloid content of the extract	3.38 ± 0.03	3.30 - 3.46
Total flavonoid content of the extract	1.81 ± 0.01	1.78 - 1.84
Total tannin content of the extract	2.19 ± 0.17	1.77 - 2.62
Total saponin content of the extract	0.31 ± 0.01	0.29 - 0.32

The values characterize the means ± SD of three replicate analyses (N=3).1: g quinine sulphate /100g extract, 2: g quercetin/100g extract, 3: g tannic acid/100g extract, 4: g diosgenin /100g extract; CI: Confidence Interval.

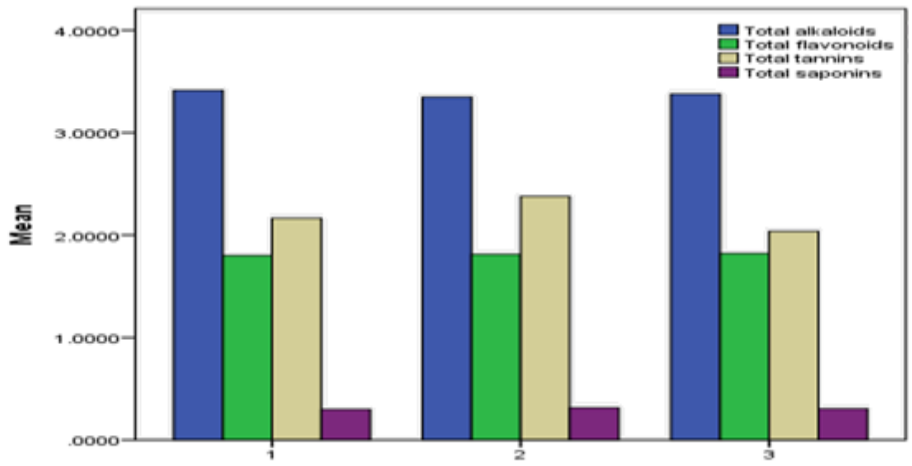

Figure1. Quantitative phytochemical analysis with Error bars: 95% CI

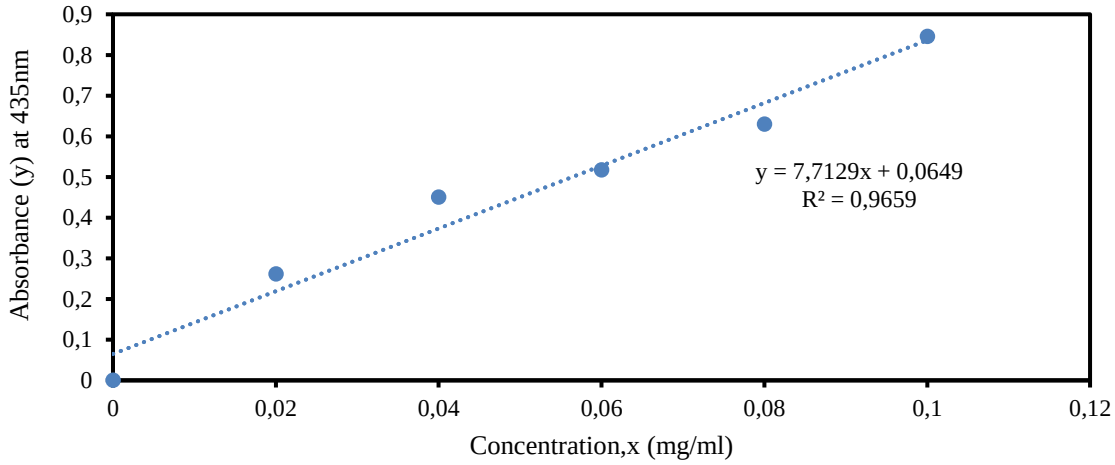

Figure 2. Standard calibration curve of quinine sulphate reference solution

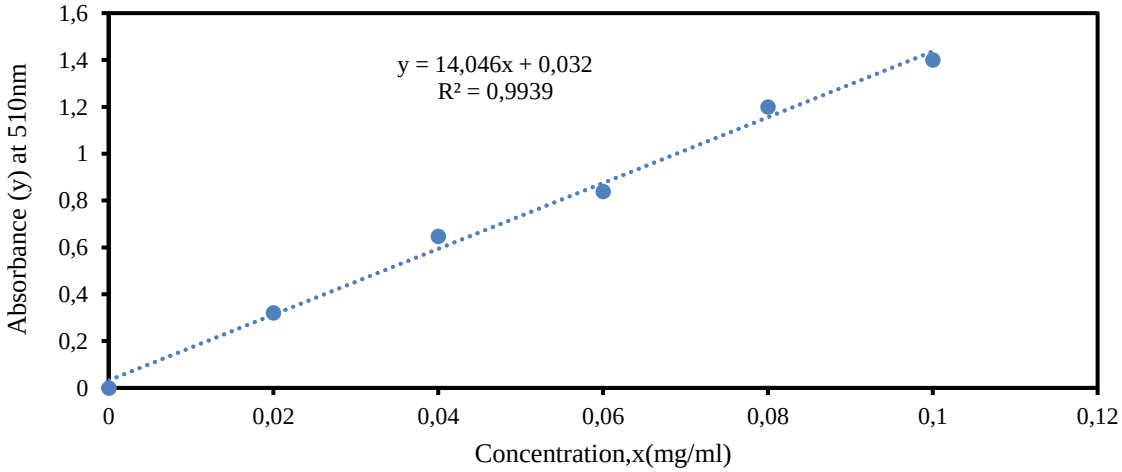

Figure 3. Standard calibration curve of quercetin reference solution

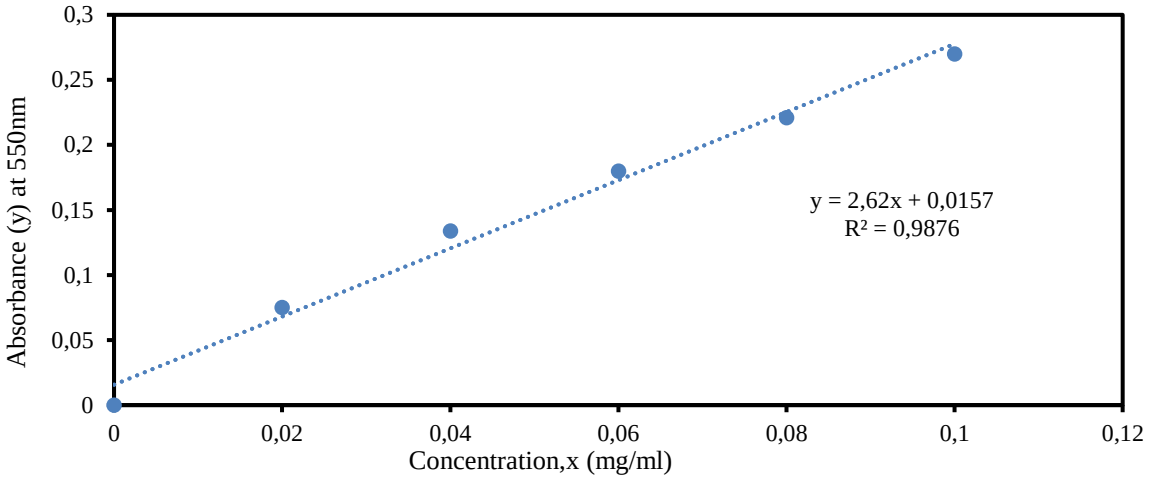

Figure 4. Standard calibration curve of tannic acid reference solution

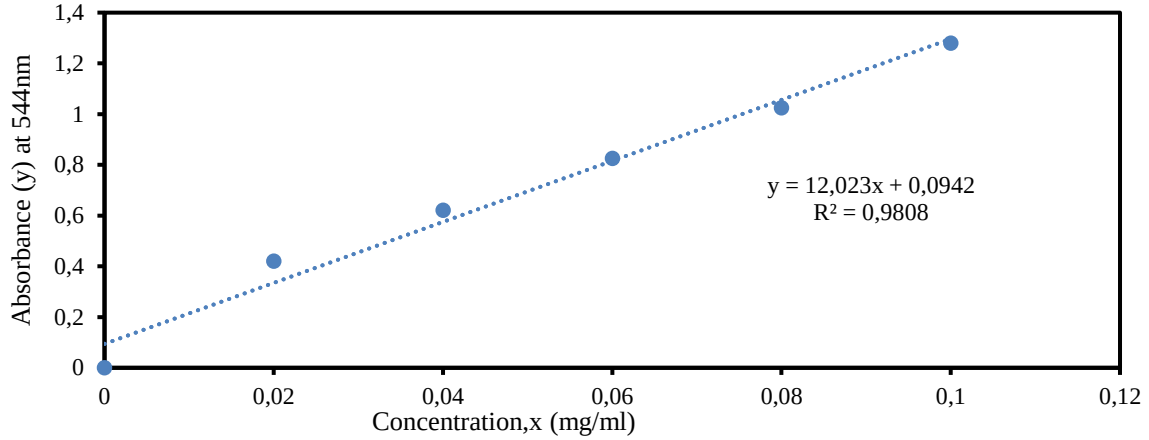

Figure 5. Standard calibration curve of diosgenin reference solution

Traditional healers use tannin-rich plants to treat a wide range of conditions, such as rheumatism, high blood pressure, wounds, diarrhea, kidney and bladder problems, and inflammatory conditions, among others [1]. Consequently, *E. tomentosa* may be useful for treating inflammation, preventing infections, and boosting antioxidant activity because it contains tannins [1] [11].

Tannins have therapeutic properties because, in addition to being able to form complexes with other biomolecules like polypeptides and carbohydrates, they also have a high level of complexity with metallic particles like iron and manganese [17]. Meanwhile, phlobatannins were not detected while tannins were present, yet they have a similar basic structure to tannins. The reason could be that phlobatannins have a more complex chemical structure than hydrolysable and condensed tannins, so any non-sensitive test should fail to detect their presence. Further studies are needed to retest and elucidate the scientific cause of the absence.

In the field of pharmacology, glycosides have long been demonstrated to be effective in the management of a variety of diseases. Cardiac glycoside, for example, has been used in dart poisons and pharmaceuticals for centuries because of its ability to treat a wide range of ailments, including cancer [8]. Researchers have found that different terpenoids and

phenylpropanoid products can treat inflammation and pain, stop blood clots from forming, and stop intracellular signaling pathways from functioning [18].

Saponins are essential since it has been demonstrated that they help lower cholesterol levels and prevent cancer. The chemotherapeutic natural saponin acts on cancer cells' cholesterol-abundant plasma tissues, stopping their multiplication [19]. Anti-cancer medications might be made from the leaves of *E. tomentosa* since they contain saponins. Saponin precursors are also used in the production of essential beneficial medications like cortisone and estrogen-based contraception [20].

Quantitative analysis carried out on four phytochemicals (alkaloids, flavonoids, saponins, and tannins) reveals that these secondary metabolites were present in different amounts in the leaves of the methanol plant extract (Table 2). Amongst the quantified phytochemicals, the alkaloid content of the leaves of *E. tomentosa* was found to be 3.38%(w/w) followed by tannin at 2.19%(w/w), flavonoid at 1.81%(w/w), and then saponin, which was found to be 0.31%(w/w). Further research needs to be done on the leaves of plants so that the structures that are liable for their numerous biological properties can be isolated, identified, characterized, and explained.

4 Conclusions

According to our findings in this study, *E. tomentosa* methanol leaf extract has the potential to serve as a foundation of valuable pharmaceuticals due to the existence of a variety of phytochemical constituents like alkaloids and terpenoids as well as cardiac glycosides, saponins, coumarins, steroids, flavonoids, tannins, among others. Thus, secondary metabolites are what give these plants their medicinal effects. This plant could potentially be used to produce synthetically better medical molecules in the future, raising the prospect of even more innovative therapeutics being developed. Though promising, further research is needed before this can become a reality.

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6 Declarations

6.1 Author Contributions

All authors contributed to the research concept and design. The laboratory analysis was performed by Mboneye Anselme under Odoma Saidi's supervision. Timothy Neeza contributed under statistical data analysis and interpretation while literature and writing were done by Mboneye Anselme under Odoma Saidi and Albert N. Onchweri's supervision.

6.2 Conflicts of Interest

No conflicts of interest have been declared by the authors.

6.3 Ethic

Not applied.

7 References

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