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Phytochemical Analysis of the Aerial Parts of *Tephrosia Elegans* with Antibacterial and Antioxidant

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(BU/UP/2021/1528)

A research report submitted to the Department of Chemistry in partial fulfilment of the requirement for the award of the degree of Bachelor of Science Education of Busitema university

May, 2024

DECLARATION

I ALOWO FLORENCE declare that this work has been done by myself. In addition, the work has not been submitted for any other degree or professional qualification. I confirm that the work is my own. My contribution and those other authors to this work have been fully acknowledged and cited according to the university policy.

SIGN ..... DATE 26th August, 2024

ALOWO FLORENCE

BU/UP/2021/1528

APPROVED AND SUPERVISED BY

DR. OWOR RICHARD ORIKO

SIGN ..... DATE 26/8/2024

DEDICATION

I dedicate this work to my sister who has also been a mother as well, APIO LAKERI who has tirelessly worked towards ensuring that I settled in school with all the necessary requirements and in time.

ACKNOWLEDGEMENT

I humbly and wholeheartedly in a special way thank my supervisor Dr. Oriko Richard Owor for the tireless work and time that he has invested during the process of my research. He has been a mentor and a very accessible person and also motivated me in several aspects. May God reward the woks of his hands.

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ABSTRACT

There has been high prevalence of skin diseases worldwide. Some of these are caused by bacteria especially staphylococcus aureus. These pathogens have also led to production of reactive molecules from the metabolism of oxygen like superoxide and hydrogen peroxide that give rise to a variety of pathological conditions among which is inflammation There are several bacterial skin treatments that have been used towards this challenge but these bacteria especially S. aureus have become resistant to drugs for example penicillin, niacinamide and many others. Plants are valuable source of biologically active molecules The phytochemical screening of the aerial parts of *T. elegans* has shown presence of most of these phytochemical components especially tannins, flavonoids, alkaloids, phenols, steroids and glycosides. The total quantification of the different components present in *T. elegans* resulted into alkaloids with 118.9 mg/g, phenols with 48.69mg/g, flavonoids with 33.34mg/g and tannins with 2.63mg/g. The antioxidant study on the methanolic extract of *T. elegans* using DPPH test was also determined and it demonstrated an antioxidant scavenging percentage of 41.87%. In addition, the antibacterial activity was also investigated using the disc-diffusion method on Nutrient Agar. In the disc-diffusion assay, the solutions of the plant extract in methanol (80%) and DMSO were dipped on sterile filter paper discs of 6mm diameter. Then the filter paper discs were placed on agar plates uniformly inoculated with *S. aureus* at 37◦C for 24 hours. A paper disc with DMSO was used as a negative control. The diameter of the clear zone surrounding the disc was used to measure the antibacterial activity of the plant extract. After 24 hours, there was no inhibition of the bacterial by the plant extract in all the plates hence *T. elegans* does not inhibit *S. aureus*.

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ACRONYMS, ABBREVIATIONS AND DEFINITION OF TERMS

DPPH: 2,2- Diphenyl-1- picrylhydrazyl

AE: Atropine Equivalent

QE: Quercetin Equivalent

AE: Gallic Acid Equivalent

CHAPTER.1 INTRODUCTION

1.1 Background of study

Some bacteria are pathogenic and cause infections leading to various diseases. Among the diseases caused by pathogenic bacteria are skin diseases particularly impetigo, cellulitis, folliculitis, carbuncles, and necrotizing fasciitis (Brodsky & Medzhitov, 2009; Vannberg et al., 2011).

It is estimated that skin diseases account for 5% of the global disease burden. Factors like poor hygiene, overcrowding, and limited access to healthcare contribute to the high prevalence of these infections in many under developed regions. Different regions face varying burdens of specific bacterial skin infections. Impetigo is common in low- and middle-income countries, affecting children disproportionately. Cellulitis is a significant concern in developed countries, often associated with chronic conditions and antibiotic resistance.(Olatunji et al., 2023). Skin diseases are regarded as neglected tropical diseases and yet they cause psychosocial problems, stigmatization, exclusion and distress [WHO 2020]. On the other hand, bacterial infections lead to several oxidant reactions which cause skin inflammations due to production of reactive oxygen species.

Bacterial infection causes the skin to be abrased, excoriated and ulcerated which result into inflammation, ruptured blisters, lesion, development of pus and skin disorders. Most of the bacterial skin infections are caused by ***Staphylococcus aureus*** gram-positive *Staphylococcus aureus, Streptococcus pyogene, Pseudomonas, Escherichia* and *Klebsiella* causing difficult-to-treat infections. However, *Staphylococcus aureus* have been found to cause several skin infections including impetigo, boils, and many others. Several antibiotics have been used in the treatment of skin infections, and some of them include penicillin, cephalosporins, clindamycin, trimethoprim-sulfamethoxazole, doxycycline, muprocin Cream or Ointment and many others. However, due to misuse and overuse of the antibiotics, these bacteria have become resistant for example *S. aureus* has become resistant to methicillin. Traditionally, medicinal plants have been used to manage bacterial skin infections such as *T. villosa, T. pupuria*, and other plants. In this study, *T. elegans* was phytochemically investigated and its antibacterial as well as antioxidant properties was evaluated.

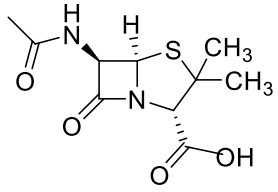

Figure 1: Penicillium

1.2 Statement of the problem

There has been high prevalence of skin diseases worldwide. Some of these are caused by bacteria especially staphylococcus aureus. These pathogens have also led to production of reactive molecules from the metabolism of oxygen like superoxide and hydrogen peroxide that give rise to a variety of pathological conditions among which is inflammation. There are several treatments that have been used towards this challenge for example penicillin, niacinamide, cephalosporins, clindamycin, trimethoprim-sulfamethoxazole, doxycycline, mupirocin Cream or Ointment and many others. However, S aureus has developed resistance to these drugs. Therefore, there is need to develop an alternative treatment for these skin bacterial infections and oxidant activity. The class of genus of Tephrosia have some plants that have shown efficacy against bacteria for example *T. puparia* was found to be effective against these bacteria (Chen et al., 2014). However, *T. elegans* has not been evaluated against these skin causing pathogens. Therefore, phytochemical screening of the aerial parts of *T. elegans* towards antioxidant and antibacterial was carried out which led to the screening of different phytochemical components.

1.3 Objective of study

1.3.1 General objective

To investigate phytochemical composition of the aerial parts of *T. elegans* antibacterial and antioxidant and herbal soap formulation.

1.3.2 Specific objectives

i. To investigate the phytochemical composition of *T. elegans*

ii. To analyse the efficacy of antibacterial and antioxidant activities of *T. elegans.*

1.4 Justification of study

Medicinal plants have been known as a great source of bioactive compounds that are important in the development of drug for example penicillin derived from *penicillium* for bacterial infections. *T. elegans* whose phytochemical composition was investigated contains alkaloids, phenols, tannin and flavonoids whose properties make it a potential resource for making new drugs.

1.5 Scope of the study

This research was purely experimental with explorative studies on bioactive components of *T. elegans*. It was majorly focused on comparative analysis of antibacterial and antioxidant components of the aerial parts of *T. elegans.*

CHAPTER.2 LITERATURE REVIEW

2.1 Description of *T. elegans*

Plants of the genus Tephrosia mainly inhabit tropical and subtropical regions with over 30 species occurring in Kenya. From many decades, plants have been used for the ailment of diseases. Traditional medicines refer to innumerable approaches such as animal- and mineral-based medicines, spiritual therapies, knowledge and beliefs in incorporating plant to treat, diagnose and prevent illness of the well-being. Most of the modern medicine currently used for various treatments has many undesirable effect and unpredictable pharmacological action; hence, the need to search for the newer drugs with lesser or no side effects is obligatory (Owor, Derese, et al., 2020).

Tephrosia genus belongs to the family Fabaceae (Leguminosae) and subfamily Paplilionaceae, which contains about more than 350 species of the genus. The plants in this genus are chiefly distributed in the regions of tropical, subtropical, and arid regions of the world. The plants are erect herbs, or it is in the form of soft or woody shrubs. Based on several studies conducted by the taxonomist, *Tephrosia* was classified into four sections, namely, *Mundulea*, *Brissonia*, *Craccoides*, and *Reineria*, out of which *Mudulea* and *Reineria* were represented in India. Later, the genus has been classified into three subgenera which includes *Marconyx* (includes *T. tenuis*), *Brissonia* (includes *T. candida*), and *Reineria* (includes rest of the species of *Tephrosia)*. Phytochemical investigations revealed the presence of a number of phytoconstituents. The bioactivity associated with the plant has been studied extensively, indicating the phytoconstituents present in the *Tephrosia* genus manifested various biological activities such as anti-diabetic, anti-ulcer, anti-diarrheal, wound healing, anti-inflammatory, insecticidal, anti-viral, anti-protozoal, anti-fungal, anti-plasmodial, and many other activities. Several literature surveys showed a very few or no reviews were available which correlates the data of phytochemical, pharmacological, and molecular properties of the genus *Tephrosia* together (Owor, Derese, et al., 2020; Samuel et al., 2019).

Many of the species have been used ethnomedicinally to alleviate diverse illnesses. Phyto-chemical investigations of some of these plants by our research group have led to the isolation of a chalcone, rotenoids, flavanonols, and flavones that are biologically active. Also, the genus Tephrosia is reported to elaborate other bioactive flavonoids such as flavanones, isoflavones, and pterocarpans. Some of these flavonoids also exhibit anti-inflammatory properties. For in-stance, genistein found in Tephrosia toxicaria reduces peripheral and central nuclear factor-κB (NF-κB) and the nitric oxide system as well as pro-inflammatory cytokine overactivation, while naringenin, common in the family Fabaceae, decreases the production of TNF-α, and apigenin inhibits TNF-α-induced NF-κB(Owor, Bedane, et al., 2020) .

2.2 The phytochemistry of *T*

Various research studies have been carried out to study the chemical constituents of the variety of plants belonging to genus *Tephrosia*. Many of the organic compounds belonging to different classes have been isolated. Among many of the organic compounds isolated, some have been used for their pharmacological properties and some of them are still unknown for their effects. It was found that flavonoids were the most commonly isolated and identified compound in the genus, the other main classes of compounds include rotenoids, terpenoids, sterols, essential

oils, and fixed oils. Not many research studies have been carried to indicate the presence of essential oil and fixed oil. For many of the taxonomists, *T. purpurea*, *T. toxicaria*, *T. candida*, *T. elata*, and *T. villosa* have been a sign of interest. Also, there are works done on the stereochemistry of the compounds, for example, a flavonoid from *Tephrosia pumila* called as Praecansone, exists in two isomers.

Many of the isolated compounds have been studied for their pharmacological actions. There are many chemical components mentioned that are not studied under the genus *Tephrosia* but the literature survey suggested their presence in other genera. For instance, Pseudosemiglabrin, Flemichapparin, Caryophellene oxide, deguelin, pongamol, and lupeol possess platelet aggregation antagonism, anti-fungal, anti-cancer, anti-convulsant, and anti-inflammatory activities, respectively. Although a lot of research has been carried out on majority of the Tephrosia genuses, no screening to find out the bioactive components has so far been carried out on *T elegans*. Therefore, a phytochemical screening of *T. elegans* is to be carried out in the laboratory to find out the bioactive components in it(Samuel et al., 2019).

2.3 The antibacterial activity of *T. elegans*

While there is limited specific research directly investigating the antibacterial activity of *T elegans*, some evidence suggests potential and warrants further research. Presence of bioactive compounds: As mentioned earlier, Tephrosia species in general are known to contain rotenoids, flavonoids, and other classes of compounds with documented antibacterial properties. Rotenoids have been shown to disrupt bacterial cell membranes, while flavonoids possess antioxidant and anti-inflammatory effects that can indirectly impact bacterial growth.

The historical use of *T. elegans* leaves as fish poison and its inclusion in arrow poisons indicate the potential presence of compounds with biocidal activity, possibly including antibacterial effects. Targeted isolation and characterization of antibacterial compounds: Identifying and isolating specific compounds responsible for antibacterial activity from *T. elegans* would further our understanding of its potential (Zhang et al., 2020).

Therefore, further research has been carried out to fully explore its potential effectiveness and safety in addressing bacterial infections and oxidant activities and the findings have been found as shown in this report.(Ha et al., 2022; Kong et al., 2016).

2.4 Antioxidant activity of *T. elegans*

The antioxidant potential of *T. elegans* is an intriguing area of research with promising indications but limited concrete evidence. Potential for antioxidant activity in *T. elegans* is indicated by: Presence of known antioxidants: Several Tephrosia species, including *T. vogelii* and *T. purpurea*, have been shown to contain flavonoids, especially apigenin, luteolin, and kaempferol. These compounds are well-documented for their antioxidant properties, scavenging free radicals and protecting cells from oxidative damage. *T. elegans* has also been traditionally used in some regions for its anti-inflammatory and wound-healing properties, which could be linked to potential antioxidant activity. Therefore, further research has been carried out to explore its potential effectiveness and safety in promoting antioxidant benefits for human health and the results are as shown in this report.

Figure 2: Tephrosia elegans aerial parts

CHAPTER.3 MATERIAL AND METHODS

3.1 Plant Material

3.1.1 Plant collection and extract preparation

Fresh leaves of all plants were collected from shrubs at Ebusbusi, Katakwi, Uganda. The GPS on the latitude 2.09484, N2◦5'41.42904, log 34.06663 and E34◦3'59.87556. The voucher specimens waw confirmed by a botanist and deposited in Busitema University Herbarium called Mrs. Carol Kawuma. The leaves were dried under shade in the laboratory for two weeks. They were ground to fine powder with an automated electric grinder. After grinding, the sample was stored in an air tight container.

3.2 Extraction

The shade dried and ground powder of the aerial parts of *T. elegans*(10g) was extracted using hot reflux extraction and concentration method with 10ml of 80%methanol at temperature of 45◦C for about 15 minutes. The extracted was then concentrated under reduced pressure to obtain the crude. (Owor et al., 2021)

3.3 Phytochemical analysis

The crude extract of the aerial parts of the *T. elegans* were dissolved in different respective solvents using the different procedures and the phytochemical analysis of the plant extracts was carried out by the standard methods provided.

3.3.1 Test for tannins

3.3.2 Ferric chloride test

Few drops of ferric chloride solution were added to 1mL of aqueous solution of the *T. elegans* crude extract (a small part of the crude was dissolved in 1mL of distilled water and filtered) using a test. A blackish precipitate indicated the presence of tannins.

3.3.3 Lead acetate test

Few drops of aqueous basic lead acetate solution were added to 1Ml of aqueous solution of the *T. elegans* crude extract using a test tube. A reddish-brown bulky precipitate indicated the presence of tannins.

3.3.4 Test for phenolic compounds:

3.3.5 Ferric chloride test

2 ml of the crude extract of *T. elegans* solution was mixed with ferric chloride solution. Formation of an intense colour indicated the presence of phenolic compounds.

3.3.6 Ellagic acid test

Few drops of 5% glacial acetic acid and 5% sodium nitrate solution were added to 2Ml of an alcoholic/ aqueous solution of the crude extract using a test tube. Formation of a brown precipitate indicated the presence of the phenols.

3.3.7 Test for saponins

2 cm^3 of the *T. elegans* crude extract in a test tube was mixed with 10 mL of distilled water then agitated for 10 minutes. Foam formation which disappears immediately indicated the absence of saponins.

3.3.8 Test for Flavonoids

3.3.9 Ferric chloride test

Few drops of neutral ferric chloride solution were added to 1 ml of an alcoholic solution of a crude extract of *T. elegans* using a test tube. Formation of a blackish red colour indicated the presence of flavonoids.

3.3.10 Lead acetate test

Few drops of aqueous basic lead acetate solution were added to 1 ml of alcoholic extract using a test tube. The appearance of a reddish-brown bulky precipitate indicated he presence of flavonoids

3.3.11 Detection of steroids

3.3.12 Salkowski test

1 ml of concentrated sulphuric acid was added to 5 ml of a chloroform solution of the crude extract (a small part of the crude extract was dissolved in chloroform and filtered) using a test tube. It was vortexed and allowed to stand for 5 minutes. Lower layer turned into red colour which indicated the presence of steroids.

3.3.13 Liebermann-Burchard test

Few drops of acetic anhydride were added to 5 ml of a chloroform solution of the crude extract followed by adding 1 ml of concentrated sulphuric acid through the wall of the test tube and allowed to stand for 5 minutes. Formation of a reddish-brown ring at the junction of the two layers and a green colour in the upper layer indicated the presence of steroids.

3.3.14 Detection of Glycosides

3.3.15 Fehling's test for glycosides.

10 cm^3 of 50% H_2SO_4 was added to 1 cm^3 of the extract in a test tube. The mixture was heated in a boiling water-bath for 15 minutes. 10 cm^3 of Fehling's solution was then added and the mixture was boiled. Formation of brick red precipitate indicated the presence of glycoside.

3.3.16 Test for alkaloids

3.3.17 Mayer's test

1 ml Mayer's reagent (potassium mercuric iodide solution) was added to 1 mL of n acidic solution of the crude extract. Cream colour precipitate indicated the presence of alkaloids.

3.3.18 Wagner's reagent

1 ml of Wagner's reagent (iodine in potassium iodide) was added to 1 ml of an acidic solution of the crude extract. The formation of a reddish brown precipitate indicated the presence of alkaloids.

3.3.19 **Test for triterpenes**.

3.3.20 Salkowski test

20mg of crude extract of *T. elegans* was added to 2ml of chloroform with few drops of conc.H_2SO_4. The absence of the reddish-brown colouration of the interface indicated absence of terpenoids.

3.4 TOTAL QUANTITATIVE ANALYSIS

This will majorly focus on determining the concentration of the compounds in the extract of *T. elegans*

3.4.1 Total flavonoid content

The aluminium chloride spectrophotometric assay was used to estimate total flavonoid content in *T. elegans*. In this method, the sample contained 1 ml of a methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution was dissolved in methanol. The samples were incubated for 1 h at 25 °C. The absorbance was determined using spectrophotometer at λ_{max} 420 nm. The samples were prepared in triplicate for each analysis and mean value of absorbance was obtained. The same procedure was repeated for the standard solution of quercetin and the calibration line is constructed. Based on the measured absorbance, the concentration of flavonoids was read (µg/ml) on the calibration line. Thereafter, total flavonoid content in a *T. elegans* was expressed in terms of quercetin equivalent (mg of QE/g of extract).

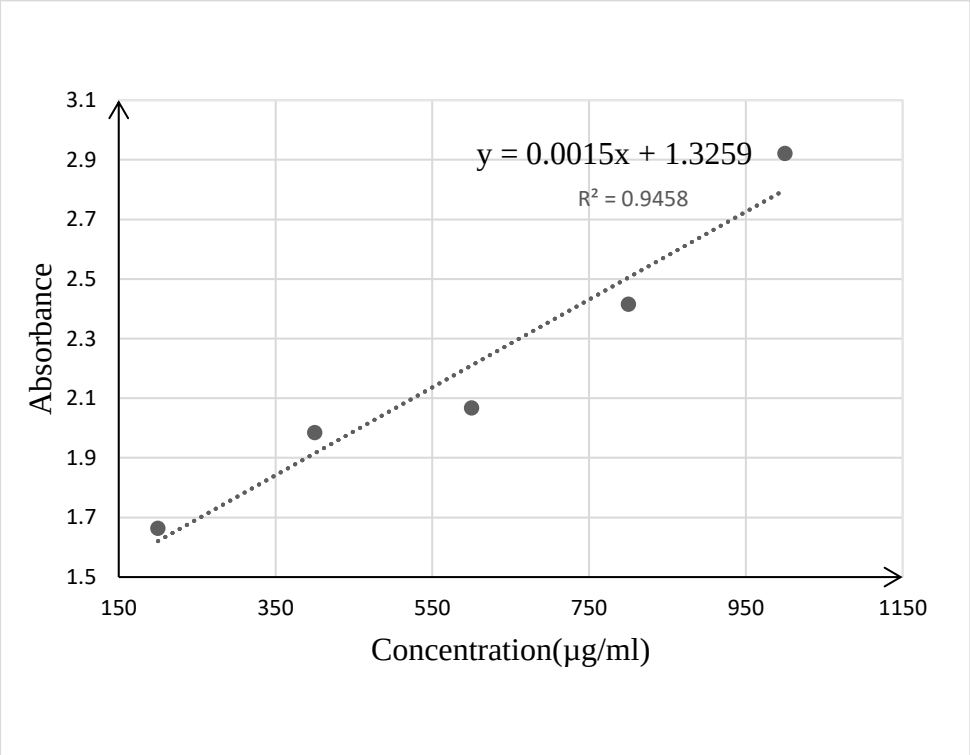

Figure 3: A calibration curve for Flavonoid

$$Total\ flavonoids = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ ample}$$

Where X = concentration of the extract in mg/ml obtained from the calibration curve

3.4.2 Total phenol content:

Folin-Ciocalteau method was used to estimate the total phenol content in aerial parts of *T. elegans*. In this method, a methanolic solution of the extract (1mg/ml) was added to 2.5 ml of 10% Folin-Ciocalteau reagent dissolved in water and 2.5 ml of 7.5% sodium carbonate. A blank was similarly prepared containing 0.5ml methanol, 2.5 ml of 10% Folin-Ciocalteau reagent dissolved in water and 2.5 ml of 7.5% sodium carbonate. The samples were incubated in a thermostat at 45^0C for 45 minutes and the absorbance was determined using spectrophotometer at λ max 292nm using a single beam UV-VIS spectrophotometer (6705 JENWAY). The same procedure was repeated for the standard solution of propyl gallate. The calibration curve will be constructed using standard propyl gallate solution prepared at concentrations of 0.2, 0.4, 0.6, 0.8 and 1mg/ml. The concentration of phenols was read (mg/ml) from the calibration curve. Thereafter total phenolic content in the plant was expressed in terms of propyl gallate equivalent (mg of PGA/g of extract).

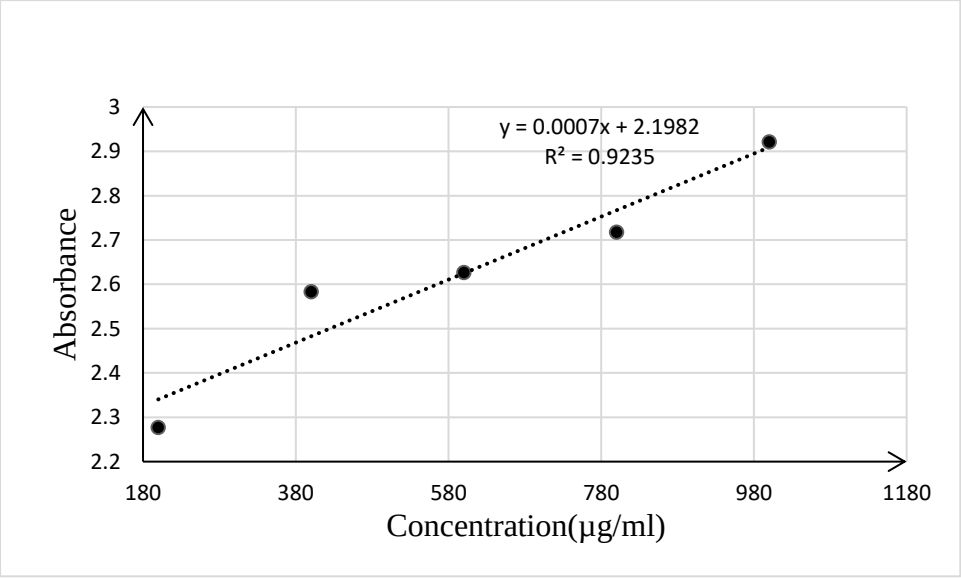

Figure 4: A calibration curve for phenols

$$Total\ phenol = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ sample}$$

3.4.3 Total tannin content

100 µL of 10 mg/mL extracts were added to a clean test tube containing 7.5 mL of distilled water. The Folin-Ciocalteau reagent (0.5 mL) was added to the mixture and vortexed thoroughly. 10mL of 35% solution of sodium carbonate ($NaCO_3$) was added to the mixture. The mixture in the test tube was transferred to 10 mL volumetric flask and the volume of the mixture made up to 10mL with distilled water. The mixture was shaken and kept at ambient temperature for 30 minutes in the dark. Tannic acid was used as a standard and reference

standard solutions (1.0- 0.2 mg/ml) were prepared. The absorbance of the solution was measured at 650nm against a blank reagent blank using a single beam UV-VIS spectrophotometer (6705 JENWAY). The calibration curve was constructed using standard tannic acid solution prepared at concentrations of (1.0, 0.8, 0.6, 0.4 and 0.2 mg/ml). The concentration of phenols was read (mg/ml) from the calibration curve. (*Screening of Phytochemicals in a Crude Plant Extract_013152*, n.d.)

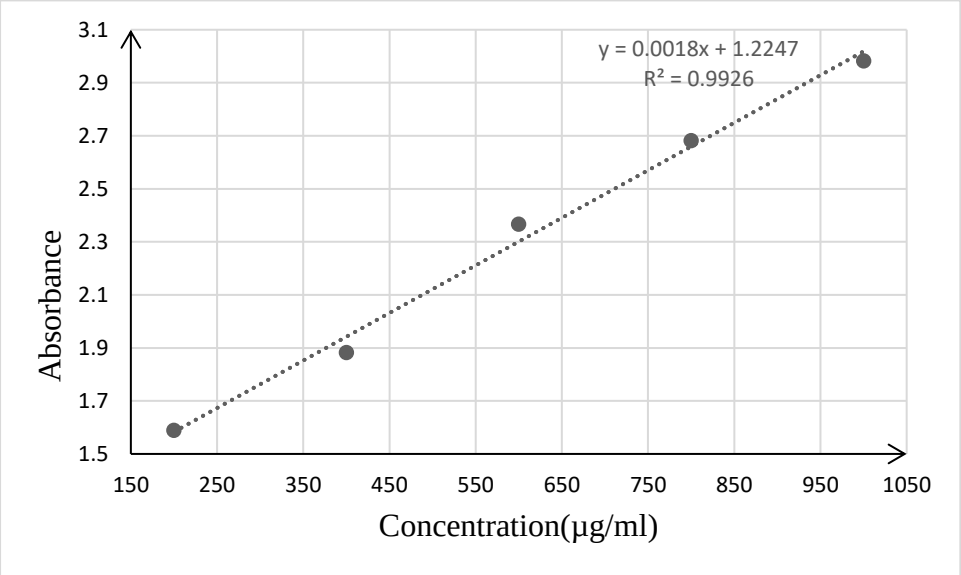

Figure 5: a calibration curve for Tannins

$$Total\ Tannins\ (\text{mg/g of the extract}) = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ sample}$$

3.4.4 **Total alkaloid content**:

A solution of 1 mg/mL of plant extract was prepared using methanol. 1 mL of 2 M HCl was added to 1 mL of methanol dissolved extracts and the resulting mixture was filtered using filter paper. The filtrate was transferred to a 250 mL separating funnel and to this solution, 5 mL of 0.1% Bromocresol green (dissolved in methanol) was added followed by 5 mL of phosphate buffer (pH 6.6). Chloroform (1 mL) was added into the separating funnel and the mixture was vigorously shaken, after which the funnel will be allowed to stand to allow the mixture to separate into different layers. The lower layer was collected in a 10 mL volumetric flask. The process was repeated with 2, 3, and 4 mL of chloroform. Atropine was used to construct a standard curve using a concentration range of 1.0–0.2 mg/ml. The absorbance of the sample and standard solutions was recorded at a wavelength of 470 nm against a reagent blank using a single beam UV-VIS spectrophotometer (6705 JENWAY). The total alkaloid content was expressed as milligram atropine equivalent/ gram of extract (mg AE/g). All the measurements were evaluated in triplicate. The calibration curve was constructed using a standard tannic acid solution prepared at concentrations of 1.0, 0.8, 0.6, 0.4 and 0.2 mg/ml.(*Screening of Phytochemicals in a Crude Plant Extract_013152*, n.d.)

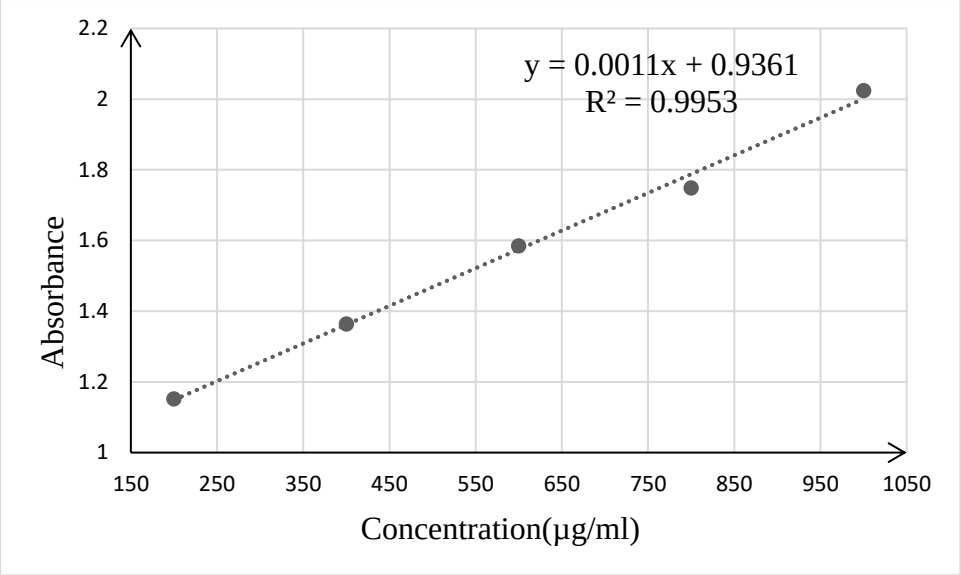

Figure 6: A calibration curve for Alkaloids

$$Total\ alkaloid\ (\text{mg/g of the extract}) = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ sample}$$

3.4.5 Determination the antioxidant activity

3.4.6 Free radical scavenging activity

Free radical scavenging activity of the plant extracts was quantified using k.. Different concentrations of the extract (1000-200 µg/mL) were prepared to a volume of 1 mL of the solution. L-ascorbic acid is used as a standard by preparing the same concentration range as the extracts. To this 1 mL solution, 2 mL of 0.2 mmol/L DPPH solution was added and vortexed thoroughly. All the prepared mixtures are left to stand in the dark for 30 min. The control solution is prepared by adding 2 mL of 0.2 mmol/L DPPH to 1 mL of distilled water. After the elapsed time, the solutions are analysed with a UV/VIS spectrophotometer. The absorbance of the solutions was read at 446 nm.

$$\%\ scavening = \left(\frac{Ac - As}{Ac}\right) * 100$$

Where Ac is the absorbance of the control solution, As is the absorbance of the extracts

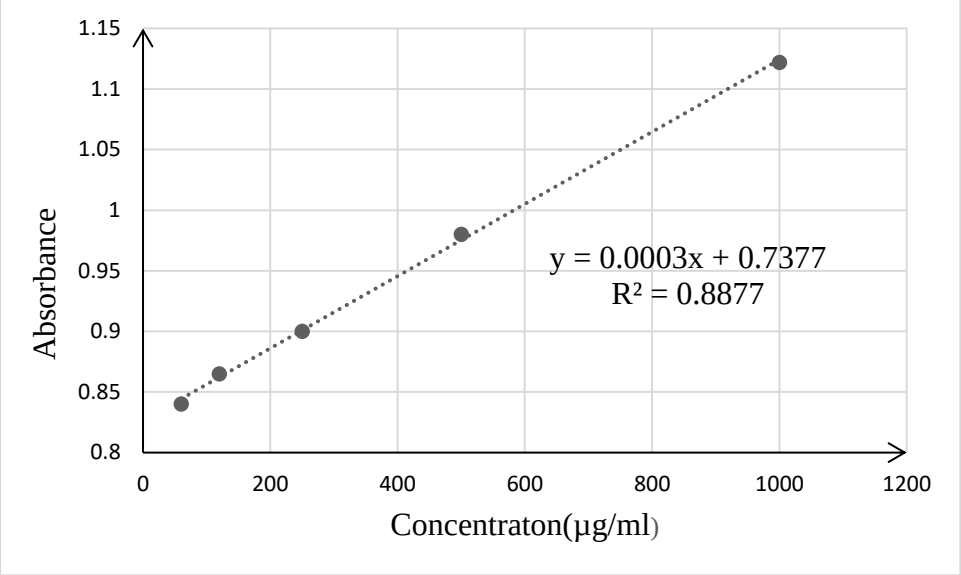

Figure 7: Calibration curve for antioxidant

$$\%\ scavening = \left(\frac{1.655 - 0.962}{1.655}\right) * 100 = 41.87\%$$

Where Ac is the absorbance of the control solution, As is the absorbance of the extracts

3.5 Antibacterial activity

Antibacterial activity of the prepared plant extract was evaluated using the disk diffusion method against S. aureus. The pure bacterial cultures were cultured on a nutrient Agar. The plates were left overnight at room temperature the inoculation of the plates with S. aureus was carried out. Sterile paper disks were place on the inoculated nutrient agars each with different concentration of the plant extract. The test plates were incubated at 37◦C for 24 hours.

Figure 8. Agar plate with disc containing plant extract

CHAPTER.4 RESULTS AND DISCUSSION OF RESULTS

4.1 Phytochemical Analysis

The phytochemical screening results showed that the methanolic extract of *T. elegans* had high concentration of alkaloid, phenolic compounds and flavonoids. The concentration of tannins and steroids was moderate and no saponins and terpenoids (Table 1).

Table 1: Phytochemical screening and quantification results

Phytochemical	**Test**	**Quantity**	**Quantified sample (mg of standard equivalent/g of crude extract)**
Alkaloid	Wagner's test	+++	118.9
	Mayer's test	+++	
Flavonoids	Ferric chloride test	++	33.34
	Lead acetate test	++	
Tannins	Ferric chloride test	+	2.63
	Lead acetate test	+	
Phenolic compound test	Ferric chloride test	+++	48.69
	Ellagic test	+++	
Steroids	Salkowski test	+	ND
	Liebermann-Bushard test	+	
Glycosides	Sulphuric acid test	+	ND
Saponins	Water and agitation	-	ND
Terpenoids	Salkowski test	-	ND
Key: +++ highly present ++ moderately present + weakly present -absent ND = Not Determined			

Following the phytochemical screening of the methanolic aerial parts of *T. elegans*, it was found that the phytochemicals present were flavonoids, tannins, phenols, alkaloids, steroids and glycosides. The total quantification of the different components contained in *T. elegans* resulted into alkaloids with 118.9 mg/g, phenols with 48.69mg/g, flavonoids with 33.34mg/g and tannins with 2.63mg/g. Similar studies on the different genus of Tephrosia showed the presence of similar phytochemical compounds for example *T. pupuria, T. villosa, T. vigolli* and many others.

The antibacterial activity of the plant extract refers to the ability to inhibit the growth of bacteria. Although the phytochemical analysis of the *T. elegans* revealed the presence of several phytochemical components like tannins, phenols, alkaloids, flavonoids, steroids and glycosides, it was found to have no inhibition on the selected bacteria. This is in coherence of similar results with different species of Tephrosia where several species showed mild or no inhibition with *S. aureus.*(Bravim dos Santos et al., 2021)

However, *T. elegans* had a great antioxidant activity of scavenging percentage of 41.87% using DPPH test. This could have been due to the presence of flavonoids and phenolic compounds which neutralize lipid free radicals and hydroperoxides from decomposing into free radicals. This information is in coherence to several articles on different Tephrosia species.

CHAPTER.5 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The aerial pats of *T. elegans* extract quantitatively contain flavonoids, alkaloids, tannins, glycosides, steroids and phenolic compounds.

However, the major phytochemical components were alkaloids, flavonoids, tannins and phenols with compositions of 118.9 mg/g, 33.34 mg/g,2.63 mg/g, and 48.69 mg/g respectively. These compounds may be responsible for the antioxidant activity. The plant extract also showed no inhibition of the selected bacteria using the disc diffusion method on Nutrient Agar after it was left for 24 hours.

5.2 RECOMMENDATION

According to the set objectives, I recommend that further studies should be carried out to isolate and characterize the bioactive components in the plant extract which could be responsible for the antioxidant activity and also many other antimicrobial properties.

I also recommend that different bacteria be tested with this plant extract since it is inactive with *S. aureus.*

CHAPTER.6 REFERENCES

Agbon, A., Ofojekwu, C., & Ezenwaka, I. (2004). Acute toxicity of water extract of Tephrosia vogelii Hook to species relevant in aquaculture ponds: rotifers, Cyclops, mosquito larvae and fish. *Journal of Applied Ichthyology*, *20*(6), 521–524.

Bharadwaj, A., Rastogi, A., Pandey, S., Gupta, S., & Sohal, J. S. (2022). Multidrug-Resistant Bacteria: Their Mechanism of Action and Prophylaxis. *BioMed Research International*, *2022*.

Blommaert, K. L. J. (1949). The plant tephrosia vogelii, hooker, as a freshwater fish poison. *Transactions of the Royal Society of South Africa*, *32*(3), 247–264.

Brodsky, I. E., & Medzhitov, R. (2009). Targeting of immune signalling networks by bacterial pathogens. *Nature Cell Biology 2009 11:5*, *11*(5), 521–526.

Chen, Y., Yan, T., Gao, C., Cao, W., & Huang, R. (2014). Natural products from the genus Tephrosia. *Molecules*, *19*(2), 1432–1458.

Gachene, C. K. K., & Wortmann, C. S. (2004). Green Manure/Cover Crop Technology in Eastern and Central Uganda: Development and Dissemination. *Green Manure/Cover Crop Systems of Smallholder Farmers*, 219–236.

Ha, N. M., Tran, S. H., Shim, Y. H., & Kang, K. (2022). Caenorhabditis elegans as a powerful tool in natural product bioactivity research. *Applied Biological Chemistry*, *65*(1).

I, N. (2014). Sub-acute Toxicity of Aqueous Extracts of Tephrosia vogelii, Vernonia amygdalina and Senna occidentalis in Rats. *Natural Products Chemistry & Research*, *2*(5).

Kong, C., Eng, S. A., Lim, M. P., & Nathan, S. (2016). Beyond traditional antimicrobials: A Caenorhabditis elegans model for discovery of novel anti-infectives. *Frontiers in Microbiology*, *7*(DEC), 1–14.

Mkindi, A. G., Tembo, Y., Mbega, E. R., Medvecky, B., Kendal-smith, A., Farrell, I. W., Ndakidemi, P. A., Belmain, S. R., & Stevenson, P. C. (2019). Phytochemical Analysis of Tephrosia vogelii across East Africa Reveals Three Chemotypes that Influence Its Use as a Pesticidal Plant. *Plants 2019, Vol. 8, Page 597*, *8*(12), 597.

Olatunji, Y. A., Banjo, A. A., Jarde, A., Salaudeen, R., Ndiaye, M., Galega, L. B., Odutola, A., Hossain, I. M., Osuorah, C., Sahito, M. S., Muhammad, B. S., Ikumapayi, N. U., Drammeh, M. M., Manjang, A., Adegbola, R. A., Greenwood, B. M., Hill, P. C., & Grant, M. A. (2023). Invasive bacterial disease in young infants in rural Gambia: Population-based surveillance. *Journal of Global Health*, *13*, 1–11.

Owor, R. O., Bedane, K. G., Openda, Y. I., Zühlke, S., Derese, S., Ong'amo, G., Ndakala, A., & Spiteller, M. (2021). Synergistic anti-inflammatory activities of a new flavone and

other flavonoids from Tephrosia hildebrandtii vatke. *Natural Product Research*, *35*(22), 4486–4493.

Owor, R. O., Bedane, K. G., Zühlke, S., Derese, S., Ong'Amo, G. O., Ndakala, A., & Spiteller, M. (2020). Anti-inflammatory Flavanones and Flavones from Tephrosia linearis. *Journal of Natural Products*, *83*(4), 996–1004.

Owor, R. O., Derese, S., Bedane, K. G., Zühlke, S., Ndakala, A., & Spiteller, M. (2020). Isoflavones from the seedpods of Tephrosia vogelii and pyrazoisopongaflavone with anti-inflammatory effects. *Fitoterapia*, *146*(May), 1–6.

Samuel, V. J., Mahesh, A. R., & Murugan, V. (2019). Phytochemical and pharmacological aspects of Tephrosia genus: A brief review. *Journal of Applied Pharmaceutical Science*, *9*(3), 117–125.

Sattley, W. M., & Madigan, M. T. (2021). Bacteriology. *Encyclopedia of Life Sciences*, *1*, 821–829.

Vannberg, F. O., Chapman, S. J., & Hill, A. V. S. (2011). Human genetic susceptibility to intracellular pathogens. *Immunological Reviews*, *240*(1), 105–116.

Young, K. D. (2007). Bacterial morphology: why have different shapes? *Current Opinion in Microbiology*, *10*(6), 596–600.

Zhang, P., Qin, D., Chen, J., & Zhang, Z. (2020). Plants in the genus tephrosia: Valuable resources for botanical insecticides. *Insects*, *11*(10), 1–18.