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Phytochemical analysis and anti-bacterial activity of the extract of the leaves of *Tephrosia villosa*

BY

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Research dissertation submitted to the department of chemistry in partial fulfillment of the requirements for the award of the degree of Bachelor of Science Education of Busitema University.

May, 202

DECLARATION

I Abbo Margret declare that this research dissertation has been composed by myself and has not been submitted for any other degree or professional qualification. I confirm that the work submitted is my own, except where work which has formed part of jointly-authored publications has been included.

Signature.....**Date**..14/5/2024.....

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ABBO MARGRET

BU/UP/2021/1509

APPROVAL

This research dissertation has been submitted for examination with my approval as his university supervisor.

Signature.....**Date** .....14/5/2024.....

DR OWOR RICHARD ORIKO

DEDICATION

This report is dedicated to my mother Ms. Atengei Jenifer, who has supported and guided me spiritually, physically, emotionally and financially in achieving my academics. This research is also dedicated to fellow chemistry students for their special time, support and cooperation exhibited for the success of my research.

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ABSTRACT

In developing countries, childhood pneumonia remains a leading cause of death in children and accounts for up to 21% of deaths in children under the age of five years. In sub-Saharan Africa, the estimated proportion of death in children aged below 5 years attributed to pneumonia is 26%. Uganda is currently ranked among the 15 countries with the highest estimated number of deaths due to clinical pneumonia. Pneumonia is caused by many microorganisms including viruses, fungi and bacteria. *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* are the most predominant cause of bacterial pneumonia. It is usually treated using antibiotics such as penicillin, amoxicillin and clarithromycin. However, pseudomonas aeruginosa has become resistant to some of the antibiotics especially clarithromycin which has got a variety of side effects such as diarrhea, stomach pain, vomiting, loss of appetite Therefore, there is need for an alternative source of effective antibiotics with minimal side effects.

Tephrosia villosa was traditionally used in Africa to manage various diseases due to the fact that it possesses various medicinal properties including the antibacterial activity. This study was to analyze the phytochemical composition of the methanolic extract of the plant and evaluate its anti-bacterial activity. Alcoholic extracts of *T. villosa* leaves were subjected to various phytochemical analysis to identify the phytochemical components present in the plant.

These phytochemicals (alkaloids, flavonoids, phenols and tannins) were then quantified using different standards whose calibration curves were used to determine the total amount of each phytochemical. Antibacterial assay of the Crude extract of *Tephrosia villosa* was carried out to investigate its effectiveness on *pseudomonas aeruginosa* bacteria. The results of the phytochemical analysis confirmed the presence of various phytochemical compounds including alkaloids, flavonoids, steroids, glycosides, saponins, tannins and phenolic compounds. Quantification results showed that the extract had a high concentration of alkaloids (144.85mg/g), moderate concentration of flavonoids (41.35mg/g) and tannins (39.09) with low concentration of phenols (14.18mg/g). Antibacterial activity of the leaves extracts of the plant material showed a clear zone of inhibition on *pseudomonas aeruginosa* of 44.44% in the lowest concentration of the sample. Further investigation should be carried to determine the specific bioactive compounds responsible for the anti-bacterial activity and against other bacterial strains like *streptococcus pneumoniae* to fully explore the antibacterial potential of *Tephrosia villosa.*

DEFINITION OF TERMS

T. villosa: Tephrosia villosa

TT: Total tannins

TA: Total Alkaloids

TP: Total phenols

TF: Total Flavonoids

WHO: World Health Organization

DF: Dilution factor

H_2SO_4: Sulphuric acid

AE: Apigenin equivalent

APE: Atropine equivalent

PGA: Propyl gallate equivalent

UV-VIS: Ultraviolet visible spectrometer

CAP: Community Acquired Pneumonia

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CHAPTER 1 INTRODUCTION

1.1 Back ground of the study

Pneumonia is an acute respiratory infection that affects the lungs causing difficulty in breathing. It is responsible for high morbidity and mortality rates among children under five years and possess a major threat to public health worldwide (Liu et al., 2016). Globally, pneumonia is the leading cause of child mortality, responsible for approximately 354,000 deaths in children under-five (Keeley & Little, 2017; Unicef, 2019).

In developing countries, childhood pneumonia remains a leading cause of death in children and accounts for up to 21% of deaths in children under the age of five years (Zar & Ferkol, 2014). In sub-Saharan Africa, the estimated proportion of death in children aged below 5 years attributed to pneumonia is 26% (Farooqui, Jit, Heymann, & Zodpey, 2015). Uganda is currently ranked among the 15 countries with the highest estimated number of deaths due to clinical pneumonia (Tramper-Stranders, 2018).

Pneumonia is caused by many microorganisms including viruses, fungi and bacteria. *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* are the most predominant cause of bacterial pneumonia (Sattar, Sharma, & Headley, 2023). It is usually treated using antibiotics such as penicillin, amoxicillin and clarithromycin (Langtry & Brogden, 1997). However, *P. aeruginosa* has become resistant to some of the antibiotics especially clarithromycin which has got a variety of side effects such as diarrhea, stomach pain, vomiting, loss of appetite (Bonvehi, Weber, Busman, Shortridge, & Notario, 2003). Therefore, there is need for an alternative source of effective antibiotics with minimal side effects.

Traditionally, plants have been used for treatment of diseases. Most of the modern medicines currently used for various treatments have many undesirable effect and unpredictable pharmacological action (Campillos, Kuhn, Gavin, Jensen, & Bork, 2008); hence there is need to search for the newer drugs with lesser or no side effect. Plants produce chemicals to protect themselves and many phytochemicals which can protect humans against infectious diseases (Samuel et al., 2019).

Tephrosia villosa shows various biological activities including the antibacterial activity (Samuel et al., 2019). However, several literature surveys showed a very few or no reviews available which correlates the data of phytochemical and anti-bacterial activity. Thus, the main purpose of this research is to identify and quantify the phytochemicals present in *Tephrosia villosa*, determining the anti-bacterial activity and formulation of herbal syrup for management of pneumonia.

1.2 Statement of the problem

Pneumonia has been one of the commonest cause of suffering worldwide among children under five years, with the developing nations carrying the highest mortality and morbidity pneumonia burden (Hernández-Mosqueda, Guerrero-Rosas, & Tobón-Tobón, 2015). 18% of all the under-five childhood death in Uganda is recognized to be due to severe pneumonia (Nantanda, Tumwine, Ndeezi, & Ostergaard, 2013). Despite several treatment options, the prevalence of pneumonia remains high among children who are under five years and therefore there is need to develop more effective treatment options. Traditional medications (local herbs) have been the option to replace the drugs because of fear of their consequences. *T. villosa* has been traditionally used to manage pneumonia due to its medicinal properties such as anti-bacterial activities hence its ability to inhibit bacteria *P. aeruginosa* which causes bacterial pneumonia. For that reason, these findings diagnostically aim at investigating the anti-bacterial activity of *T. villosa*.

1.3 Objectives of the study

1.3.1 General objective

To investigate the phytochemical compositions and analyze the anti-bacterial activity of the leaves extract of *T. villosa.*

1.3.2 Specific objectives

1. To determine the phytochemical compositions of the leaves of *T. villosa.*
2. To analyze the efficacy of anti-bacterial activity of the leaves of *T. villosa.*

1.4 Justification

T. villosa needs to be used as an alternative medicine because the bacteria have become resistant to the antibiotics and therefore since this plant is used traditionally for the management of diseases, a bioactive compound can be identified to treat pneumonia. More so, many drugs have been developed from medicinal plants including aspirin from the back of willow tree plant, quinine from the bark of cinchona tree.

CHAPTER 2 LITERATURE REVIEW

2.1 The nature of *T villosa*

T. villosa commonly known as fish poison in the traditional system and belongs to the family Fabaceae, species villosa and genus *Tephrosia* which is the major group of angiosperms (flowering plants) that comprises of more than 350 species widely distributed in the regions of tropical and subtropical countries of the world. It is a multibranched perennial bushy herb, 0.3-1.3m tall ,white stem with hairy compound leaves which is commonly found on sandy soil.(O Nondo et al., 2011)

Figure 1: Showing the leaves of *T. villosa* plant

Traditionally, *T. villosa* has been used in different parts of the world; ethno botanical studies reveal that root powder and paste has been used for stomach ache, fever and typhoid, used for various skin disorders in Karnataka, used for dental pain in Tamil Nadu, used for treatment of dropsy and enlargement of viscera. In Ethiopia, it is used for respiratory tract disorders and literature survey reveals that *T. villosa* has been found to poses anti-microbial property, anti-diabetic, anti-oxidant, used as potential bio-insecticide and green corrosion inhibitor

Despite being reported to be toxic to live- stock and fish, aqueous extract of *T. villosa* leaves is an erect herb used widely in traditional Indian medicine as remedy in the treatment of diabetes mellitus. (Madhusudhana et al., 2010) In Africa, the herb is used as green manure to improve the soil and as well the ethanolic extracts from leaves, fruits and roots of *T. villosa* are used for larvicidal and antimicrobial activity (O Nondo et al., 2011).

Since the herbal medicine is in demand due to its fewer associated side effects, the genus *Tephrosia* is extensively used for the treatment of large number of diseases in traditional medicines. Plants produce various chemicals to protect themselves; but recent studies proved that many

phytochemicals can also protect humans against infectious diseases. The present study was conducted to identify and characterize the phytoconstituents and gas chromatography–mass spectroscopy (GCMS) analysis of petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and aqueous extract of leaves of *Tephrosia villosa.*

Phytochemical investigations of *Tephrosia villosa* showed the presence of a number of phytoconstituents and its bioactivity has been studied extensively. *Tephrosia villosa* manifested various biological activities including the antibacterial activity (Samuel et al., 2019).

It has been noted that phytochemical screening of leaf extract of *T. villosa* revealed the presence of alkaloids, flavonoids, triterpenoids, saponins, glycosides, steroids, tannins, phenols and fixed oils in methanolic extract which is followed by aqueous extract. The phytoconstituents of plant leaves are scientifically proved for their anti-diabetic, anti-ulcer, anti-anxiety, capable of reducing liver toxicity due to their antioxidant properties and many other pharmacological activities. Flavonoids are the most abundantly isolated and identified compounds in the genus (Obbalareddy et al., 2013). In quantitative analysis, the highest amount of phenolic content was obtained in aqueous extract, tannin in petroleum ether, and flavonoids and saponins in ethyl acetate.

Indigenous and traditional medicines make extensive use of natural products and derivatives of natural products and provide more than half of all medicines consumed today throughout the world and ethno pharmacology plays an important role in the discovery of new biologically active compounds. According to World Health Organization (WHO) more than 80% of the world's population uses plants for the treatment of their diseases. The genus Tephrosia, belonging to the Leguminosae family, is a large pan tropical genus of more than 350 species, many of which have important traditional uses

It has also been noted that infectious diseases and disorders caused by pathogenic microorganisms like bacteria, viruses, fungi, and protozoa and multicellular have been managed by modern medicines. These medicines are primarily from plant origin which have less toxicity and their importance is being in both developed and developing countries.

2.2 The phytochemistry of *T. villosa*

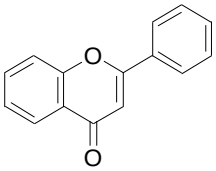
A great variety of plants belonging to genus *Tephrosia* have been studied for their chemical constituents and pharmacological activities (Sheik Nasar et al.). Different classes of organic compounds have been isolated of which some have been tested for their biological activities and some still unknown for their effect. It should be noted that flavonoids are the most abundantly

isolated and identified compounds in the genus (Obuya, 1988). Phytochemical investigations have revealed the presence of glycosides, steroids, flavonoids, phenols, tannins, saponins and alkaloids (Touqeer, Saeed, & Ajaib, 2013). Six polar solvents such as petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and water extract of the leaves of *T villosa* were tested for qualitative and quantitative phytochemical analysis (Nisha, Kiruthika, Vanitha, & Kalimuthu, 2021).

Petroleum ether revealed the presence of steroids, triterpenoids and fixed oil, ethyl acetate contains steroids, triterpenoids, glycosides and fixed oils, chloroform extract revealed the presence of alkaloids, tannins, steroids and glycosides (Sadaf et al., 2022). Ethanol extract was found to contain alkaloids, flavonoids, triterpenoids and fixed oil, methanol extract revealed the presence of alkaloids, flavonoids, tannins, steroids, triterpenoids, glycosides, gum and mucilage and fixed oils and water extract contain alkaloids, flavonoids, tannins, triterpenoids, saponins, gum and mucilage and fixed oils (Agnello, Naveen, Deepa, & Sivasubramanian, 2011).

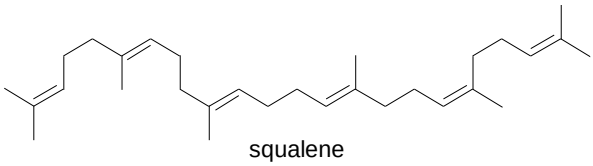
However, there is a variation of compounds in different parts of *T. villosa* and this has been attributed to environmental factors.

Some of the structures of the isolated compounds are as below,



flavone

Figure 2: structure of flavone



squalene

Figure 3: Structure of Squalene

heptacosane

Figure 4: Structure of heptacosane

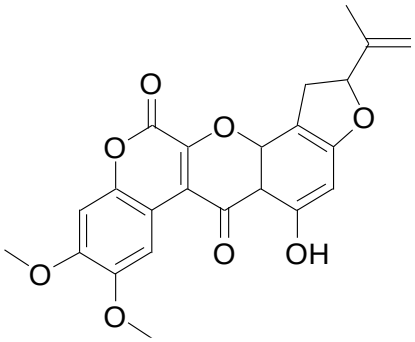


Figure 5: structure of Villosone

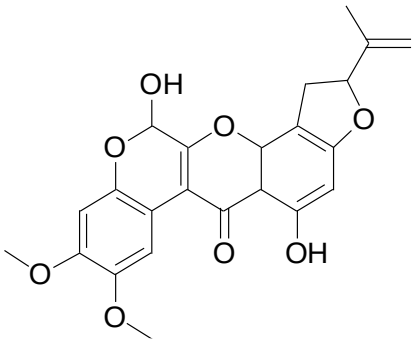


Figure 6: structure of villol

2.3 The anti-bacterial activity of *Tephrosia villosa*

Tephrosia villosa was found to possess anti-bacterial activity. The methanolic extract from the leaves was tested against Pseudomonas aeruginosa (Egharevba et al., 2019). The extracts were subjected to the minimum inhibitory concentration agar dilution method (Touqeer et al., 2013). The phytochemical analysis of the organic extracts confined the presence of alkaloids, tannins, saponins, phenols, glycosides, steroids and flavonoids which show high anti-bacterial activity (Wondimu, 2019). It is verified that the results of the methanolic extract of the stems and leaves of *Tephrosia villosa* presented the highest zone of inhibition against the bacteria pseudomonas aeruginosa by agar well diffusion method (Egharevba et al., 2019).

The predominant pathogen in CAP is pseudomonas aeruginosa which accounts for about two-thirds of all cases of bacterial pneumonia hence its inhibition by the methanolic extracts of *Tephrosia villosa* reduces on the increasing cases (Rukenya, 2014).

CHAPTER 3 MATERIAL AND METHODOLOGY

3.1 Plant material

Fresh leaves of the plant of *Tephrosia villosa* were collected from its natural habitat from Ongongoja village, Katakwi district $N2^{0}04^{1}46.578^{11}$ $E33^{0}56^{1}00.0276^{11}$ latitude 2.079605 longtitude 33.933341. It was identified and verified at Makerere University Herbarium by Ms. Carol Kawuma, a lecturer in Biology department at Busitema University Nagongera Campus and a voucher specimen was 8/2/2023. Only the leaves were used for the study. The leaves were dried in the shade. The shade dried plant samples were ground into powder using an electric motor and the powder of the sample was stored in a container.

3.2 Organic extraction (OE)

About 30g of the shade dried and ground powder of the leaves of *Tephrosia villosa* was put in a clean beaker and Dichloromethane and Methanol were added in the ratio of (1:1 v/v) at room temperature ($25^{O}C$). The mixture was filtered and the filtrate was concentrated under reduced pressure on a rotary evaporator to obtain crude.

3.3 Phytochemical analysis/tests

The organic extracts of the leaves of *Tephrosia villosa* were subjected to various phytochemical tests to identify the various active components in *Tephrosia villosa* as stated in the literature. The tests are as follows:

3.3.1 Detection of Flavonoids (Ferric chloride test)

To 1 ml of an alcoholic solution of a crude extract (dissolve a little part of crude extract in methanol and filter) was added few drops of solution in a test tube. Formation of blackish red color indicated the presence of flavonoids.

Lead acetate test:

To 1 ml of an alcoholic solution of a crude extract in the test tube (dissolve a little part of crude extract in methanol and filter) was added few drops of aqueous basic lead acetate solution. The appearance of a reddish-brown bulky precipitate indicated the presence of flavonoids.

3.3.2 Detection for alkaloids (Wagner's test)

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

3.3.3 Detection of Tannins (Ferric chloride test)

To 1 ml of aqueous solution of crude extract (dissolve a little part of the crude extract in 1 ml of distilled water and filter) in a test tube was added few drops of ferric chloride solution. A blackish precipitate indicated the presence of tannins.

3.3.4 Detection of Terpenoids

Fresh plant material was treated with 5 mL of 1% aqueous hydrochloric acid for about 4 hours. Then the crude extract obtained was treated with 1 mL of Trim-Hill reagent (10 mL of acetic acid, 1 mL of 0.2% copper sulphate in water and 0.5 mL of concentrated hydrochloric acid) in a test tube followed by heating in a water bath. The appearance of a blue color indicated the presence of diterpenoids while green color indicated the presence of monoterpenoids.

3.3.5 Detection of steroids (Salkowiski test)

To 5ml of chloroform solution of crude extract of *T. villosa* was added 1ml of concentrated sulphuric acid. The appearance of a red color indicated the presence of steroids.

3.3.6 Detection of saponins

To 2mL of the extract of *T. villosa* in the test tube was added 10ml of distilled water and shaken vigorously for 10 minutes to obtain a stable froth. Formation of a persistent froth on warming confirmed the presence of saponins.

3.3.7 Detection of Glycosides (Sulphuric acid test)

To 1 mL of concentrated sulphuric acid was added 1mL of aqueous solution of the crude extract of *T. villosa* (dissolve a little part of the extracts of *T. villosa* in 1mL of distilled and filter) and the mixture was allowed to stand for 2 minutes. The formation of reddish color indicated the presence of glycosides.

3.3.8 Detection of phenols (Ferric chloride test)

To 5ml of an alcoholic solution of the crude extract of *T. villosa* in a test tube was added 1ml ferric chloride solution. The formation of an intense color indicated the presence of phenols.

3.4 Total quantity of the phytochemicals present

3.4.1 Total flavonoid content

The total flavonoids content in *Tephrosia villosa* crude extract was determined using aluminum chloride spectrophotometric method. Apigenin was used as a standard and flavonoid content

determined as apigenin equivalent. From the standard apigenin solution, the concentrations (0.45, 0.225, 0.1125 & 0.05625 mg/ml) were prepared in methanol. 100 µl of each of the apigenin dilution was mixed with 1500 µl of distilled water followed by 100 µl of 5% sodium nitrate solution and allowed to stand for 6 minutes. Then 150 µl of 10% aluminum chloride solution was added then allowed to stand for 5 minutes after which 200 µl of solution of 1M sodium hydroxide solution was added. The absorbance of this reaction mixture was measured at λ max 416 nm using single beam UV-VIS spectrophotometer. The same procedure was repeated for methanolic extract solution of *Tephrosia villosa.* All measurements were performed in triplicate for each analysis. The total flavonoids content was determined from the linear equation of a standard curve prepared with apigenin and expressed as mg/g Apigenin equivalent (AE) of the dry extract of *T. villosa.*

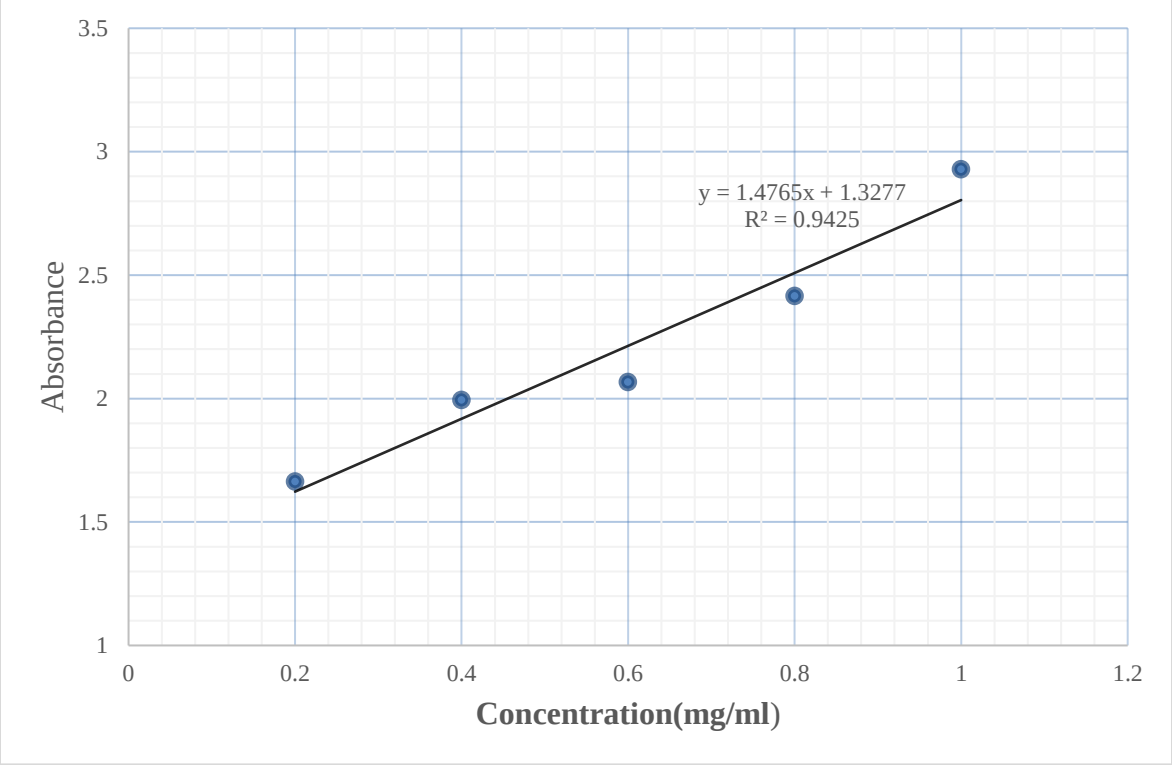


Figure 2: Calibration curve for determination of TF content

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

3.4.2 Total alkaloid content

A solution of 1 mg/ml of plant extract was prepared using dimethyl sulfoxide (DMSO). 1 ml of 2M HCl was added to 1 ml of DMSO 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 was allowed to stand to allow the mixture to separate into different layers. The lower layer is 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 1.0–0.0625 mg/ml. The absorbance of the sample and standard solutions was recorded at a wavelength of 470 nm against a reagent blank. The total alkaloid content was expressed as milligram atropine equivalent/ gram of extract (mg APE/g). All the measurements were evaluated in triplicates.

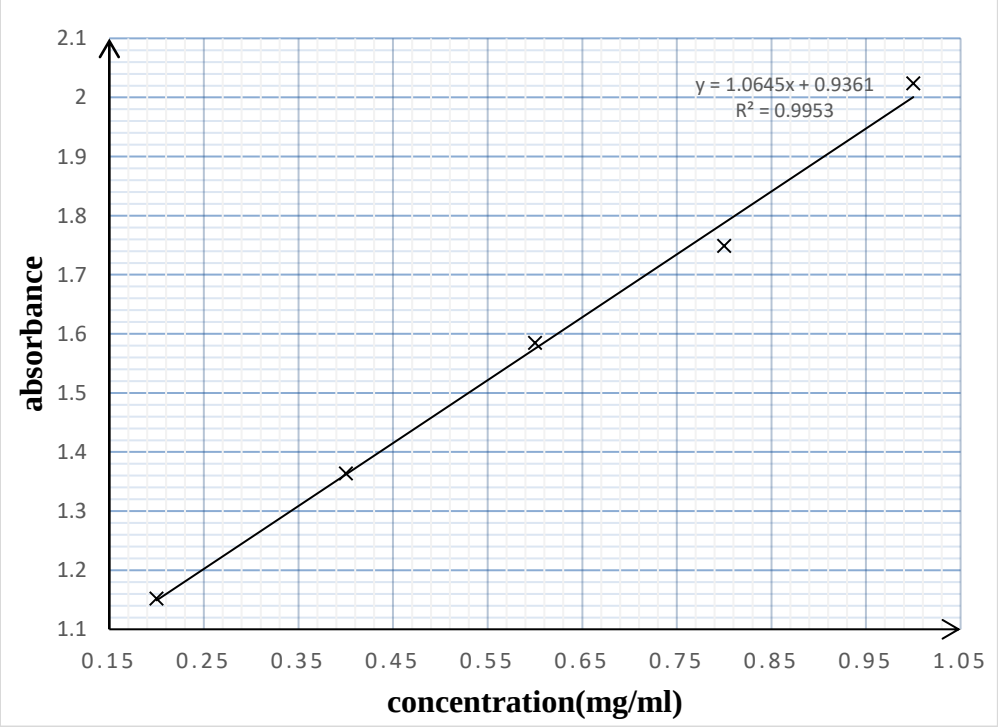


Figure 3: Calibration curve for the determination of TA content

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

3.4.3 Total tannins content

100 µL of 10 mg/mL extract was added to a clean test tube containing 7.5mL distilled water. The Folin-Ciocalteau reagent (500 µL) 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 was filled 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 (31.3- 500 mg/ml) were prepared. The absorbance of the solution was measured at 322nm against a blank reagent a single beam UV-VIS spectrophotometer. The calibration curve was constructed using standard tannic acid solution prepared at concentrations of (31.3, 62.5, 125.0, 250.0 and 500 mg/ml). Tannin content will be expressed as milligram tannic acid equivalent/gram of extract (mg TA /g).

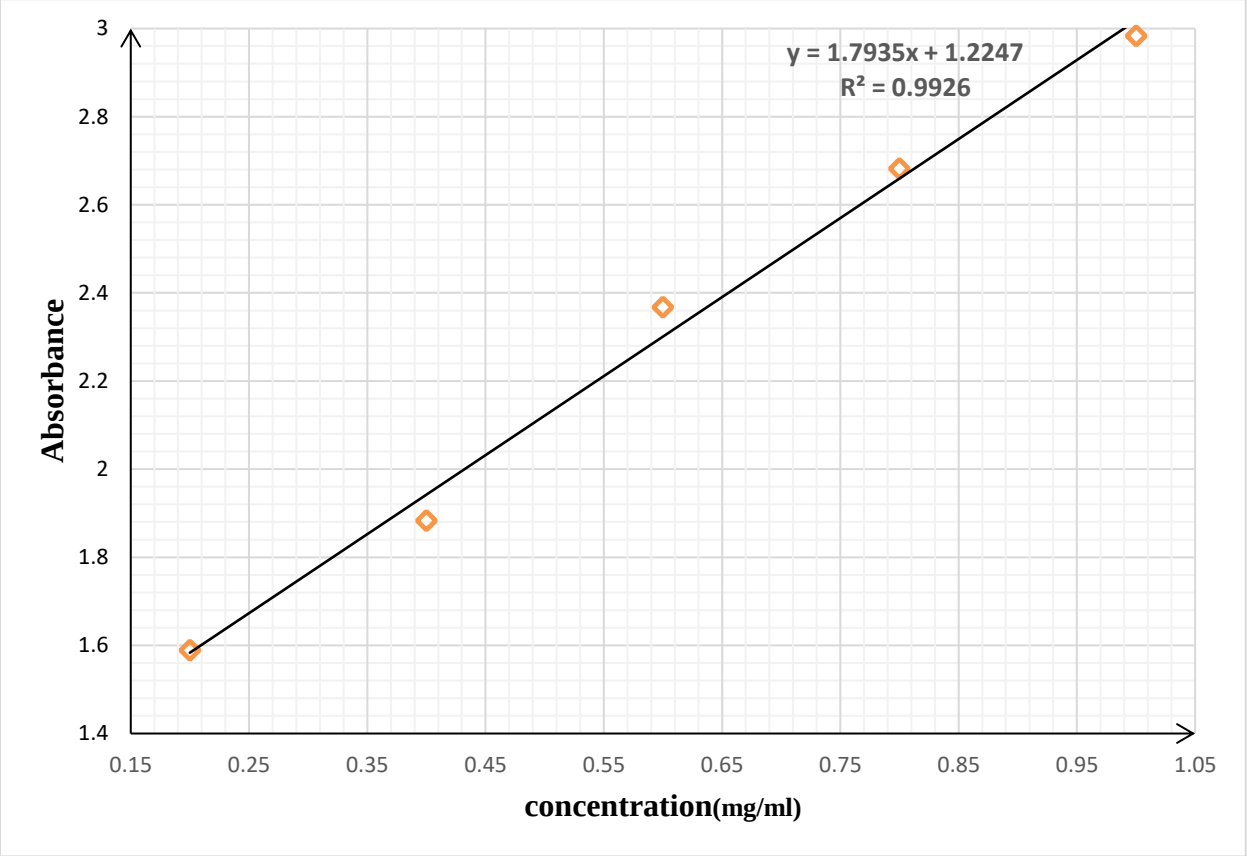


Figure 4: Calibration curve for determination of TT Content

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

3.4.4 Total phenolic content

Folin-Ciocalteau method was used to estimate the total phenol content in a pulverized sample of *Tephrosia villosa* leaves. In this method, a methanolic solution of the extract (1000 µg/ml) was added to 2500 µg of 10% Folin-Ciocalteau reagent dissolved in water and 2500 µg of 7.5% sodium carbonate was added. A blank was similarly prepared containing 500 µg methanol, 2500 µg of 10% Folin-Ciocalteau reagent which was dissolved in water and 2500 µg of 7.5% sodium carbonate. The samples were incubated in a thermostat at 45^0C for 45 minutes and their absorbance determined using spectrophotometer at λ max 765nm using a single beam UV-VIS spectrophotometer. The same procedure was repeated for the standard solution of propyl gallate. The calibration curve was constructed using standard propyl gallate solution which was prepared at concentrations of 31.25, 62.50, 125.00, 250.00 and 500 µg/ml. The concentration of phenols was read (µg/ml) from the calibration curve. Thereafter total phenolic content in the plant was expressed in terms of propyl gallate equivalent (mg of PGA/mg of extract).

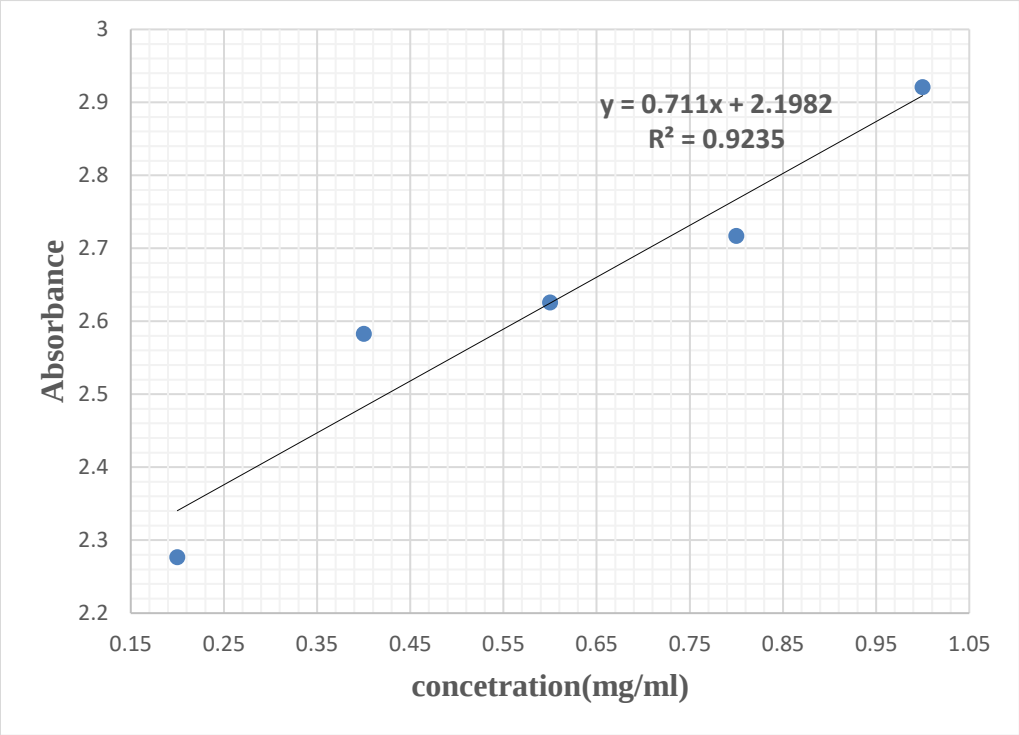


Figure 5: Calibration curve for determination of TP content

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

3.5 Anti-bacterial assay

Procedures;

Disc diffusion method was used and required the following procedures;

A standard inoculum from bacterial culture was prepared by; choosing well-isolated colonies, creating a bacterial suspension (inoculum) and finally standardized the bacterial suspension using McFarland standards.

The bacterial suspension was inoculated on MacConkey and Nutritive agar as growth mediums for disc diffusion.

Disc soaked in sample extract (antimicrobial) was added and then incubation of the plates for 24 hours to allow disc diffusion.

The zone of inhibition was measured and recorded in terms of percentage rate of inhibition and finally interpretation of antimicrobial susceptibility test results was done.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 The phytochemicals investigated

The phytochemical investigation of the extracts of the leaves of *T. villosa* revealed the presence of alkaloids, flavonoids, steroids, tannins, saponins phenols and glycosides. Total quantification showed a high alkaloid content, moderate tannins and flavonoids, and low phenols Content as shown in table 1.

Table 1: Results of selected phytochemical of organic extract of the leaves of *T. villosa*

Phytochemical	**Tests**	**Quantity present**	**Quantified amount (mg/g of crude extract)**
Flavonoids	Ferric chloride	+++	41.4
	Lead acetate	++	
Tannins	Ferric chloride	++	39.1
Saponins	Foam formation	+++	Nd
Steroids	Salkowski's test	+++	Nd
Phenols	Ferric chloride	+	14.2
Glycosides	Sulphuric acid	+++	Nd
Alkaloids	Wagner's reagent	+++	144.9
Terpenoids	Trim-Hill reagent	-	Nd

Key: strongly present +++, moderately present ++, weakly present +, absent -, Nd= not determined.

In this study, the leaves of *Tephrosia villosa* contained very high amounts of alkaloids 144.9 mg/g, moderate amounts of flavonoids 41.2 mg/g, moderate amounts of tannins 39.1 mg/g and with low amounts of phenols 14.2 mg/g.

The high concentration of alkaloids could be attributed to its potential medicinal properties such as anti-inflammatory, antimicrobial and anti-oxidant effects(Shukla, Desai, & Modi, 2020).

The moderate flavonoid content in the plant t significantly contributes to its antibacterial activity.

Flavonoids have various proven medicinal properties such as antioxidant, anticancer, anti-inflammatory, antibacterial, and antiviral properties(Ben, Sivanadanam, & Gnanasekaran, 2014).

Moderate content of tannins suggests that the plant may have a moderate level of astringency. From other studies of the leaves of *T. villosa*, the quantification of various secondary metabolites was evaluated and total phenolic was observed to be (22.3 mg/g) in aqueous extract, tannins were (3.4 mg/g) in petroleum ether extract, whereas the total flavonoids were (26.3 mg/g) and saponins (23.8 mg/g) were obtained in ethyl acetate extract(Darojati, Murwanti, & Hertiani, 2022). From this research, it shows that phenolics and tannins content were high in the methanolic extracts of T. villosa and there comprise of different medicinal properties including the anti-bacterial activity(Nisha et al., 2021).

4.2 The anti-bacterial assay

Figure 7: anti-bacterial results

Table 2 Results of the antibacterial activity of organic extracts of the leaves of *T .villosa* against pseudomonas aeruginosa bacteria

Concentration of the sample	Rate of inhibition	Percentage of inhibition

(mg/ml)	(mm)	(%)
2.00	4	44.4
1.00	3	37.5
0.50	2	28.6
0.25	1	16.7

The anti-bacterial activity of the leaves extract of *Tephrosia villosa* was carried out on *Pseudomonas aeruginosa* using agar diffusion disc method at different concentrations of 2 mg/ml, 1 mg/ml and 0.5 mg/ml and 0.25 mg/ml. These showed a clear zone of inhibition against *P. aeruginosa* hence a promising profile for the management of pneumonia. However, in other study, the crude ethanolic extract of *T. villosa* were tested for inhibitory against *P. aeruginosa* and *S. aureus* by Agar well diffusion method where the well diameter was 6 mm. The zone of inhibition was found out to be 28.6% for *P. aeruginosa* and 35.8% for *S. aureus* in the concentration of 0.2 mg/well. This shows that the results obtained from this research are nearly compatible with results obtained by other researchers(Kanu, Ezeocha, & Ogunka, 2018).

CHAPTER 5 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The findings of this study indicate that *T. villosa* contains various phytochemicals including flavonoids, alkaloids, tannins, phenols, steroids, glycosides and saponins which support its traditional use in medicine and these bioactive compounds could be responsible for its anti-bacterial activity evidenced by inhibition of the bacteria growth.

5.2 RECOMMENDATION

From the findings of this study, it is recommended that there is need to formulate the herbal remedy from the leaves of *Tephrosia villosa* for the management of pneumonia and further investigation to determine the specific bioactive compounds responsible for the anti-bacterial activity is needed.

REFERENCES

Agnello, X., Naveen, J., Deepa, C., & Sivasubramanian, C. (2011). Seminar abstract. *International Journal of Nutrition, Pharmacology, Neurological Diseases, 1*(Suppl 1), S10-S30.

Ben, C., Sivanadanam, V., & Gnanasekaran, G. (2014). Comparative phytochemical screening and antimicrobial efficacy studies on two endemic species-Jatropha maheshwarii Subr. & Nayar and Jatropha villosa Wight. *Journal of Pharmacognosy and Phytochemistry, 3*(4), 213-219.

Bonvehi, P., Weber, K., Busman, T., Shortridge, D., & Notario, G. (2003). Comparison of clarithromycin and amoxicillin/clavulanic acid for community-acquired pneumonia in an era of drug-resistant Streptococcus pneumoniae. *Clinical drug investigation, 23,* 491-501.

Campillos, M., Kuhn, M., Gavin, A.-C., Jensen, L. J., & Bork, P. (2008). Drug target identification using side-effect similarity. *Science, 321*(5886), 263-266.

Darojati, U. A., Murwanti, R., & Hertiani, T. (2022). Sterqulia quadrifida r. br: a comprehensive review of ethnobotany, phytochemistry, pharmacology and toxicology. *J Pharm Sci, 1*, 2.

Egharevba, G. O., Dosumu, O. O., Oguntoye, S. O., Njinga, N. S., Dahunsi, S. O., Hamid, A. A., . . . Priyanka, U. (2019). Antidiabetic, antioxidant and antimicrobial activities of extracts of Tephrosia bracteolata leaves. *Heliyon, 5*(8).

Farooqui, H., Jit, M., Heymann, D. L., & Zodpey, S. (2015). Burden of severe pneumonia, pneumococcal pneumonia and pneumonia deaths in Indian states: modelling based estimates. *PloS one, 10*(6), e0129191.

Hernández-Mosqueda, J. S., Guerrero-Rosas, G., & Tobón-Tobón, S. (2015). Los problemas del contexto: base filosófica y pedagógica de la socioformación. *Ra Ximhai, 11*(4), 125-140.

Kanu, A. N., Ezeocha, C. V., & Ogunka, N. P. (2018). A review on bioactive compounds of yam varieties for human disease management. *Asian Food Sci J, 1*(4), 1-10.

Keeley, B., & Little, C. (2017). *The State of the Worlds Children 2017: Children in a Digital World*: ERIC.

Langtry, H. D., & Brogden, R. N. (1997). Clarithromycin: a review of its efficacy in the treatment of respiratory tract infections in immunocompetent patients. *Drugs, 53,* 973-1004.

Liu, L., Oza, S., Hogan, D., Chu, Y., Perin, J., Zhu, J., . . . Black, R. E. (2016). Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. *The Lancet, 388*(10063), 3027-3035.

Nantanda, R., Tumwine, J. K., Ndeezi, G., & Ostergaard, M. S. (2013). Asthma and pneumonia among children less than five years with acute respiratory symptoms in Mulago Hospital, Uganda: evidence of under-diagnosis of asthma. *PloS one, 8*(11), e81562.

Nisha, K. J., Kiruthika, M. L., Vanitha, A., & Kalimuthu, K. (2021). Various Solvent Effects on Phytochemical Screening and Gas Chromatography–mass Spectroscopy Analysis of Tephrosia Villosa Leaf Extract Mass. Asian Pac. *J. Health Sci, 8*(3), 158-167.

Obuya, W. (1988). *Isolation and chemical characterization of flavonoids From tephrosia interructa and tephrosia linearis.* University Of Nairobi,

Rukenya, Z. M. (2014). *Phytochemical screening, antimicrobial activity and acute toxicity of Aloe turkanensis.* University of Nairobi,

Sadaf, H. M., Bibi, Y., Ayoubi, S. A., Safdar, N., Sher, A., Habib, D., . . . Ahmed, W. (2022). Extraction, separation and purification of bioactive anticancer components from peganum harmala against six cancer cell lines using spectroscopic techniques. *Separations, 9*(11), 355.

Sattar, S. B. A., Sharma, S., & Headley, A. (2023). Bacterial pneumonia (nursing). In *StatPearls [Internet]*: StatPearls Publishing.

Sheik Nasar, I., Aisha, A., Kumar, M. N., Suryawanshi, H. P., Bisht, R., Gupta, S., . . . Khalid, M. Phytoconstituent and Pharmacological screening for anti-diabetic activity of Tephrosia villosa.

Shukla, A., Desai, K., & Modi, N. (2020). In vitro antioxidant and antimicrobial potential of Sterculia urens Roxb. root extract and its bioactive phytoconstituents evaluation. *Future Journal of Pharmaceutical Sciences, 6*, 1-11.

Touqeer, S., Saeed, M. A., & Ajaib, M. (2013). A review on the phytochemistry and pharmacology of genus Tephrosia. *Phytopharmacology, 4*(3), 598-637.

Tramper-Stranders, G. A. (2018). Childhood community-acquired pneumonia: a review of etiology-and antimicrobial treatment studies. *Paediatric Respiratory Reviews, 26*, 41-48.

Unicef. (2019). *For every child, every right: The Convention on the Rights of the Child at a crossroads*: Unicef.

Wondimu, G. (2019). *Phytochemical analysis and antibacterial activities of organic solvent extracts of Calpurnia aurea seed, root bark and root.*

Zar, H. J., & Ferkol, T. W. (2014). The global burden of respiratory disease—impact on child health. In (Vol. 49, pp. 430-434): Wiley Online Library.