

## Evaluation of the anti-bacterial activity of aqueous leaf extract of *Phyllanthus amarus* on *Streptococcus pyogenes* for the treatment of tonsillitis

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### ABSTRACT

*Phyllanthus amarus* belongs to the *Euphorbiaceae* family, it is a tiny herb that is known for its medical benefits and used widely globally. It is also an essential plant in the Indian Ayurveda medical system, and is used to treat stomach, genitourinary, liver and kidney problems and disorders. It is an astringent with bitter, stomachic, diuretic, febrifuge, and antiseptic in gonorrhoea, menorrhagia, and other genital disorders, the entire plant is employed in eastern Asia for the treatment of infections. It helps with gastritis, diarrhea, dysentery, intermittent fevers, scabies, ulcers, and wounds. The present study aimed at addressing its activity against *Streptococcus pyogenes* for the treatment of tonsillitis and also its potential as a possible source of new antimicrobial product for tonsillitis. Distilled water (maceration method) was used in the extraction of aqueous leaf extracts of *Phyllanthus amarus*. Different phytochemical screening techniques were used to identify the phytochemicals present in the aqueous leaf extract of *Phyllanthus amarus*. The Agar well diffusion and broth dilution test methods were used to test for the in-vitro anti-bacterial activity of the aqueous leaf extract of *Phyllanthus amarus* against *Streptococcus pyogenes*. The percentage yield of *Phyllanthus amarus* aqueous leaf extracted was 13.88% (83.3mg). From the results of this research *Phyllanthus amarus* could be a good source for future drug developments, from the aqueous leaf extract of *Phyllanthus amarus* in order to address the issue of need for new antibiotics due to the alarming burden of antimicrobial resistance. **Keywords:** *Phyllanthus amarus* aqueous leaf extract, Tonsillitis, *Streptococcus pyogenes*.

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### INTRODUCTION

*Streptococcus pyogenes*, often known as Group A *Streptococcus* (GAS), is a Gram-positive, facultative coccus that causes pharyngitis, tonsillitis, scarlet fever, cellulites, erysipelas, rheumatic fever, and post-streptococcal infections in humans for example, Necrotizing fasciitis and glomerulonephritis [1-5]. Tonsillitis is an inflammation of the palatine tonsils that is usually caused by an infection either bacterial or viral infection [6-8]. It can be either acute or chronic in nature [9-12]. The bacteria *Streptococcus pyogenes* causes bacterial tonsillitis and pharyngitis.

Because of its prevalence and recurrence, tonsillitis is a public health issue [13-16]. Tonsillitis can cause consequences that are either local, regional or universal in nature. *Streptococcus pyogenes* infection prevalence study indicated that Group A-hemolytic *Streptococcus* (GAS), also known as *Streptococcus pyogenes*, causes 600 million instances of acute pharyngitis and tonsillitis each year around the world [17-22]. *Streptococcus pyogenes* RHD and tonsillitis are identified late in Uganda and other low-resource settings because poor hospital reporting system and high use of

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home herbal remedies, with 85 percent of new diagnoses coming at the time of a cardiac problem [23-26].

The *Euphorbiaceae* family's *Phyllanthus amarus* is a tiny herb well known for its medical benefits and widely used globally as an essential plant in the Indian Ayurveda medical system. There has been a growing interest in the medicinal properties of natural products all around the world. It is said that the remedy for all crippling human disorders and diseases can be discovered in nature's pharmacy among the world's flora [27-29]. *Phyllanthus amarus* is a herb native to central and southern India that can reach a height of 30-60cm. All components of the plant are used for medicinal purposes. *Phyllanthus* species can also be found in other countries, such as China (e.g. *P. uminaria*). Research on the plant has revealed that the leaves and complete plants are commonly used to cure gonorrhoea, jaundice, rickets, and other ailments asthma [30-38].

But there's still no study done showing the antibacterial activity of *Phyllanthus amarus* on *Streptococcus pyogenes*. Traditional medicine is a well-known supplement to formal medical care in Sub-Saharan Africa, as evidenced by the study [39-45]. To improve primary prevention efforts of pharyngitis and tonsillitis, a

#### AIM OF THE STUDY

The aim of this research was to determine the anti-bacterial properties of an aqueous leaf extract of *Phyllanthus amarus* on

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better understanding of health-seeking behavior for sore throat both within and outside the formal medical system is critical. In Northern Uganda, a prospective mixed-methods investigation on the use of traditional medicines for sore throat was incorporated within a wider epidemiological study of tonsillitis. Children with tonsillitis symptoms were asked about their recent usage of traditional medicines as well as their utilization of health services for sore throat [46-50]. Of the one hundred children, the use of crude drugs was reported by 100 percent of the 20 parents who witnessed the traditional medicine use. In 52 percent of patients who were seen by a traditional medicine provider, they reported of being prescribed penicillin for their treatment but opted for traditional medicines. In northern Uganda, the use of traditional medicines among parents whose children have sore throat symptoms is popular, and it is routinely utilized in conjunction with diagnostic services provided by the formal healthcare system. Collaboration with traditional medicine practitioners could be a valuable resource for developing effective primary preventive and care measures for tonsillitis and lowering the worldwide burden of *Streptococcus pyogenes* infection [51-56].

*Streptococcus pyogenes* for the treatment tonsillitis.

#### METHODOLOGY

##### Study design

An in-vitro experimental study to determine the minimum inhibitory concentration and the minimum bacterial

concentration of the aqueous leaf extract of *Phyllanthus amarus*.

##### STUDY AREA

The experiment was done in Kampala international university-western campus microbiology laboratory and in the

Pharmacognosy lab because of the availability of trained personnel and needed equipment for the experiment.

##### PLANT COLLECTION, IDENTIFICATION AND OBTAINING AN AQUEOUS LEAF EXTRACT

*Phyllanthus amarus* were collected during the month of November 2022. *Phyllanthus amarus* was collected from Buhura, Bushenyi-Ishaka municipality, and it was identified by a taxonomist. Fresh leaves were then obtained from the whole plant of *Phyllanthus amarus*. It was then initially

cleaned with distilled water and the leaves were crushed proportionally to fine product using a mortar and pestle after shade drying. The powder weighed 655g after crushing and stored in a beaker and covered with aluminum foil. The container was kept in a dry, cool place before further

The powdered sample (600g) was then soaked in 3 liters of water in a 3 different plastic containers each containing 200g of the powdered leaf product in 1L of water and sealed tightly with the help of aluminum foil paper wrapped nicely around the opening of the container, so that the soaked material would not get spilled off while shaking and stirring. Furthermore, the soaked sample was left for 48 hours, with an intermittent shaking at regular intervals at room temperature

with an aid of a laboratory shaker, so that it will soak thoroughly. On the third day the soaked sample was filtered. Initially the mixture was filtered using a clean cotton cloth which was sterilized before use. Afterwards the filtrate collected was further filtered through Whatman filter paper. Later the filtrate was evaporated in an incubator at 37°C for 3 days. The final extract was an oily and sticky brown cream. On obtaining the extract, it was weighed and percentage yield calculated using the formula below

$$\begin{aligned} \% \text{ yield} &= \frac{\text{weight of extract}}{\text{Weight of powder}} \times 100\% \\ &= 83.3\text{g} / 600\text{g} \times 100 = 13.88\% \end{aligned}$$

The container of the crude extract was then marked with a marker written "aqueous leaf extract of *Phyllanthus amarus*". These crude extract was further used for the experimental test to see the

antibacterial activity and phytochemical identification of an aqueous leaf extract of *Phyllanthus amarus* and then stored in a refrigerator at a temperature range of (2°-8°C).

#### PHYTOCHEMICAL IDENTIFICATION

##### a) Test for alkaloids

To 3 drops of Wagner's reagent was added to a quantity (5 ml) of the extract.

Formation of reddish brown precipitate indicated presence of alkaloids.

##### b) Test for flavonoids

To 3 ml of extract, 1 ml of NaOH was added. Observation of yellow

color indicated the presence of alkaloids.

##### c) Test for saponins (Frothing test)

To 2 ml of the extract was placed in a test tube then 2 ml of distilled water were added, then, shaken vigorously. There was

no formation of froth which indicated absence of saponins.

##### d) Test for tannins

2 drops of 5% FeCl<sub>3</sub>, was added in 1ml of extracts. Observation of green precipitate

was taken as positive test for presence of tannins.

##### e) Salkowski's test for steroids

To 1ml of the extract, was added 5 drops of conc. H<sub>2</sub>SO<sub>4</sub>. Observation of red color

was taken as evidence for presence of steroids.

##### f) Fehling's test for glycoside

A quantity of (10 ml) of 50% H<sub>2</sub>SO<sub>4</sub> was added to 1cm<sup>3</sup> of extract in a test tube. Then mixture was heated in a boiling water bath for 15 minutes. 10 ml of Fehling's

solution was added then boil the mixture was boiled for five minutes. Formation of a brick red precipitate was taken as evidence for presence glycosides.

##### g) Test for triterpenoids.

A quantity (10 ml) of the extract was dissolved within 1ml of chloroform, 1ml of Acetic Anhydride was added followed with

addition of 2ml of concentrated sulphuric acid Formation of reddish violet color indicated the presence of triterpenoids.

##### h) Test for amino acids

To 1ml of the extract, then few drops of Ninhydrin reagent were added. Appearance of purple color was carefully

observed to indicate the presence of amino acids.

**i) Test for Phenols (Ferric chloride test)**

2 mg of extract was dissolved in 4 ml of distilled water and few drops of 10% FeCl<sub>3</sub> were added. Appearance of blue or green

color was observed to check for the presence of phenols.

**j) Test for Phlobatannins (hydrochloric acid test)**

2mg of extract was dissolved in 4 ml of distilled water and boiled with 1% aqueous hydrochloric acid. Deposition of a red

precipitate was observed as evidence for the presence of phlobatannins.

**Antimicrobial Susceptibility Test (Agar Well Diffusion Method)**

**Preparation of Mueller Hinton Agar**

**A) For *Streptococcus pyogenes*.**

9.5g of the powdered media was dissolved in 250ml of distilled water and sterilized in an autoclave at a temperature of 121°C at 100kpa (15psi) atmospheric pressure for 15 minutes. The sterilized agar was then stirred gently while being heated using heat from a Bunsen burner

and then poured on sterilized culture plates and blood agar was added, allowed to solidify and was incubated at 37°C overnight 24 hours to observe the sterility of the culture media, there was no growth on surface of the culture plate of Mueller Hinton Agar.

**B) Preparation of inoculum**

The Mueller Hinton Agar plate was sterile since there was no growth hence, was taken and *Streptococcus pyogenes* bacteria were sub cultured in this plate aseptically using a sterile wire loop by streaking from

a previously cultured media that was obtained from sore throat swap. Freshly cultured bacteria that were incubated 48 hours before the test. Inoculum was made by surface spreading on surface of agar.

**C) Agar-Well diffusion**

Five discs of about 10mm diameter were aseptically cut out from the inoculated plates using the sterile cork borer allowing at least 30mm between adjacent wells and the edge of the petri dishes. The first well was filled with 250mg/ml of the plant extract. The second well was filled with 500mg/ml of the plant extract. The third well was filled with 1000mg/ml of the plant extract each concentrations of extract were then added into the wells. The fourth well had 10µg/ml phenoxymethyl penicillin which was applied as positive control and. The fifth well was filled with 1ml of distilled water as negative control.

Plates were kept in an upright position in an incubator to allow the extracts to diffuse into the agar. The plates were incubated for 24 hours in the incubator at 37°C and observed for zone of inhibition (mm) around the wells. The diameters of the zones of inhibition of both the plant extract and the controls were measured using a metric ruler. The zone of inhibition is the diameter of the area of no growth of the organism from the well filled with plant extract or Phenoxymethyl penicillin antibiotic. A diameter of inhibition greater or equal to 7mm is indicative of susceptibility to the extract.

**Measurement of minimum inhibitory concentration**

MIC is defined as the lowest concentration of a compound/extract/drug that completely inhibits the growth of the microorganism in 24 hour. Broth dilution method was used to determine the MIC of aqueous leaf extract of *Phyllanthus amarus* against *Streptococcus pyogenes*. The bacteria were inoculated in the serial dilution of ten tubes of *Phyllanthus amarus* extract with 1mg/ml of brain heart infusion (BHI) broth. Ten test tubes labeled 1 to 10 were each filled with 1ml of the

Brain Heart infusion (BHI) with a concentration of 1g/ml.

1ml of the extract with a concentration of 1000 mg/ml was introduced to the first test tube with broth and mixed thoroughly to give the concentration of 500 mg/ml in test-tube 2, then 1ml of the mixture was transferred to test tube 3 and mixed to give the concentration of 250 mg/ml. The 1ml of the solution was again picked from test tube 3 and transferred test tube 4 to give the concentration of 125 mg/ml. The 1ml of 125 mg/ml was then picked and

transferred to test tube 5 to give the concentration of 62.5 mg/ml after thorough mixing. The 1ml of 62.5 mg/ml was picked and transferred to test tube 6 and mixed thoroughly to give the concentration of 31.25 mg/ml. Then 1ml of 31.25 mg/ml was picked again and it was transferred to test tube 7 and mixed properly to give the concentration of 15.625 mg/ml. then 1ml of 15.625 mg/ml was picked and transferred to test tube 8 and it was then mixed thoroughly to give the concentration of 7.8125mg/ml and then from test tube 8, 1ml of mg/ml was picked and transferred and mixed thoroughly to give the concentration of

3.906 mg/ml in test-tube 9. Finally, 1ml from test-tube 9 was then removed from test tube 9 and discarded. Two controls were prepared as follows; Control one had 1ml of BHI plus bacteria without the extract in order to find out whether the broth supports the growth of the bacteria. Control 2 had only 1ml of BHI and with no bacteria and extract. This was to find out whether the broth was contaminated by other organisms. Thereafter, the tubes were incubated at 37°C for 24 hours. After incubation, the tube with the least concentration showing no microorganism turbidity was considered as the MIC of the *Phyllanthus amarus* aqueous leaf extract.

**Determination of the Minimum Bactericidal Concentration (MBC)**

Using the MIC test tubes, a loop full of the mixture from each of the test tubes with no visible growth of bacteria after 24 hours of incubation was cultured on freshly prepared Muller-Hinton agar by the streak plate method and then incubated at 37°C

for 24 hours. The plates were then examined for any colony growth. The least concentration of the extract with no visible colony growth was considered as the minimum bactericidal concentration.

**STATISTICAL ANALYSIS**

The data that was gathered was compiled using MS Office Excel. Statistical analysis was done using SPSS version 21 software package (SPSS statistical for windows, version 21.0 Chicago: SPSS Inc.). ANOVA test was performed to compare different concentrations of the aqueous leaf extract

of *Phyllanthus amarus* and results were measured as mean ±SD. A p-value less than<0.05 was considered to be statistically significant and p<0.001; highly significant.

**ETHICAL CONSIDERATIONS**

Ethical approval was sought from the Research and Ethics Committee for biosafety of Kampala International University-Western Campus (KIU-WC). Permission to use the microbiology laboratory was attained from the head of department of Kampala International University. Disposal of used cultured plates and inoculums tubes were done in a

way that was directed by the laboratory technician and that ensured safety of the environment by packing all the used culture plates, inoculums tubes into an autoclave bags and sterilized by autoclaving at 121°C for about 20mins. Then later the waste media was disposed of in waste bins and some were destroyed by incineration or heating.

**RESULTS**

**Yield of Extract**

The percentage yield was calculated to be 13.88 %.

**Table 1: Percentage extractive yield from *Phyllanthus amarus* aqueous leaf extract using distilled water**

Weight of powdered plant material(g)	Weight of plant material+ container(g)	Weight of container alone(g)	Weight of extract(g)	Percentage yield (%)
600	708.7	108.7	83.3	13.88

**Phytochemical screening of the aqueous leaf extract of *Phyllanthus amarus***

The table below displays the phytoconstituents of *Phyllanthus amarus*

**Table 2: Phytochemical constituents of the aqueous leaf extract cream**

Phytochemical constituent	Results
Alkaloids	+
Flavonoids	+
Phenols	+
Saponins	+
Tannins	+
Steroids	-
Glycosides	-
Triterpenoids	+
Amino acids	-
Phlobatinins	-

KEY: + means presence of phytochemical constituent, - means not detected Alkaloids, Phenols, flavonoids, saponins, triterpenoids and tannins were present

**Antimicrobial susceptibility test (Agar well diffusion method)**

By measuring the diameter of the inhibitory zones in mm after 24 hours of incubation at 37°C and doing so three times in triplicate, the susceptibility of test

*Streptococcus pyogenes* was ascertained. The results were reported as mean± SD and are presented in table 3 below.

**Table 3: Mean inhibition zone diameter of extract of extract against test organisms.**

Test compounds	Test micro-organism	P-value
	<i>S.pyogenes</i>	
Extract 1000 mg/ml	13.3±0.667	< 0.0001
Extract 500 mg/ml	11.5± 0.707	< 0.0001
Extract 250 mg/ml	9±0.000	< 0.0001
Phenoxymethyl penicillin (10µg/ml)	27±0.000	< 0.0001

**Test applied**-two way ANOVA, with Turkey’s multiple comparisons test. Confidence level and level of significance were set at 95% and 5% respectively

**Determination of Minimum inhibitory concentration (MIC) of *Phyllanthus amarus* aqueous leaf extract against *Streptococcus pyogenes*.**

Conventionally when the test microbe is susceptible to the test extract, the MIC is usually performed. In this study, the MIC was performed using the tube dilution method .The starting concentration was

1000mg/ml and two fold dilution was done for up to nine test tubes giving concentrations from 1 to 9 of 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.81 mg/ml



and 3.91 mg/ml respectively. The results are shown in the table 4 below.

**Table 4: Minimum inhibitory concentration (MIC) of *Phyllanthus amarus* aqueous leaf extract against *Streptococcus pyogenes*.**

Concentrations(mg/ml)										
Organism	1000	500	250	125	62.5	31.25	15.625	7.81	3.91	MIC
<i>Streptococcus pyogenes</i>	-	-	-	-	+	+	+	+	+	125

**KEY, + means growth/turbidity, - means no growth/turbidity**

Measurement of minimum bactericidal concentration (MBC) of *Phyllanthus amarus* aqueous leaf extract against *Streptococcus pyogenes*. After establishing the minimal inhibitory concentration, the minimal bactericidal concentration was established by inoculating the

microorganism with various amounts of extract that produced no turbidity (no growth) on culture media, and the plates were then incubated for 48 hours. The results are displayed in the following table.

**Table 5: Minimum Bactericidal Concentration (MBC) of Phyllanthus amarus aqueous leaf extract against Streptococcus pyogenes.**

Concentrations(mg/ml)										
Organism	1000	500	250	125	62.5	31.25	15.625	7.81	3.9	MBC
<i>S.pyogenes</i>	-	-	-	+	+	+	+	+	+	250

KEY, + means growth/turbidity, - means no growth/turbidity



**Figure 1: Showing the zones of inhibition of the different experimental treatments**

- A- 250mg/ml of aqueous leaf extract
- B- 1000mg/ml of aqueous leaf extract
- C- 500mg/ml of aqueous leaf extract
- ve-Negative control (1ml of distilled water)
- +ve-Positive control (10mcg/ml of phenoxymethyl penicillin)



## DISCUSSION

The antimicrobial assay results revealed a distinctive zone of inhibition around *Streptococcus pyogenes* with a mean zone of inhibition diameter in (mm) of  $13.3 \pm (0.667)$  at a concentration of 1000 mg/ml,  $11.5 \pm (0.707)$  at a concentration of 500 mg/ml, and 9.0000 at the concentration of 250 mg/ml, the aqueous leaf extract of *Phyllanthus amarus* demonstrated activity against *Streptococcus pyogenes*. The bactericidal activity of *Phyllanthus amarus* aqueous leaf extract at 1000 mg/ml, 500 mg/ml, and 250 mg/ml differed statistically significantly ( $p=0.0005$ ). The outcomes also showed a significant difference ( $p<0001$ ) between the extracts and the negative control (distilled water), which had no inhibitory zone diameter at all concentrations against *Streptococcus pyogenes* (0.0mm). With an inhibition zone diameter of 27.000 mm for phenoxymethyl penicillin (10 g/mL), the antibacterial activity of the plant extract concentrations were significantly ( $p <0.0001$ ) lower than that of the positive control. The antibacterial activity of plant extract was  $13.3 \pm (0.667)$  at a concentration of 1000 mg/ml,  $11.50 \pm (0.707)$  mm for plant extract at 500 mg/ml, and 9.000 mm for plant extract at 250 mg/ml. This may be because, in comparison to a pure positive control at low concentration, the crude extract may still retain certain contaminants and impurities that may probably be lowering its activity. Therefore, purification is very important because the presence of some ingredients which reduce the activity of the bioactive components will be eliminated, thereby concentrating the active component hence, increasing the potency of the active components. The phenoxymethyl penicillin (10 $\mu$ g/mL) (positive control on *Streptococcus pyogenes*) showed a higher zone of inhibition diameter of  $27 \pm 000$  on *Streptococcus pyogenes* compared to the plant extract at all concentrations used in the study. This was statistically significant ( $p <0.0001$ ). Generally *Phyllanthus amarus* aqueous leaf extract had higher activity against *Streptococcus pyogenes* when the concentrations were increased. This was showed by the increase in the zones of

inhibition as the concentrations were increased; this result is in agreement with the report of [20, 26,39] which states that the higher the concentration of antibacterial substance, the higher it shows an appreciable zone of inhibition. In this present study, the minimum inhibitory concentrations of the aqueous leaf extract of *Phyllanthus amarus* that inhibited the growth of *Streptococcus pyogenes* was 125mg/ml (test tube 1/8). The Minimum Bactericidal Concentration of the extracts was 250 mg/ml for *Streptococcus pyogenes*. The phytochemical analysis of the *Phyllanthus amarus* aqueous leaf extract showed that it includes active substances like alkaloids, phenols, flavonoids, saponins, Triterpenoids and tannins. These elements may be the cause of the antibacterial activity of the *Phyllanthus amarus* aqueous leaf extract. Different methods are used by these secondary metabolites to exert their antibacterial action. According to the report by Okolo et al. the antimicrobial activity of *Phyllanthus amarus* is due to the presence of flavonoids like (quercetin), tannins, alkaloids(phyllanthin and hypophyllanthin) that act mainly by attacking the cell walls of the bacterial and thus disrupting the cell wall integrity. A study done by Hyde et al. demonstrated that some of the tested Gram positive bacteria like *Staphylococcus aureus*, *P.aeruginosa* and *B.subtilis* were more susceptible to the plant extract of *Phyllanthus amarus* than the Gram negative bacteria. This possibly explains why our extract has strong antibiotic activity against the test microorganisms. Additionally, alkaloids like securing have been shown to have a toxic effect against the cells of foreign organisms. These activities have been extensively studied for their potential use in the reduction and elimination of foreign microbes, which may account for the study's finding that the test organisms were highly susceptible to antibiotics (*Streptococcus pyogenes*). Additionally, flavonoids have been said to have anti-inflammatory and antibacterial properties.

## CONCLUSION

This study has demonstrated that *Phyllanthus amarus* aqueous leaf extract has in vitro action against the *Streptococcus pyogenes* employed in this experiment, calling for additional in vivo clinical investigations to ascertain the precise doses and its efficacy in real-world applications. It is encouraging to see that *Phyllanthus amarus*, a widely accessible local cure, can replace common

conventional antibiotics in the treatment of *Streptococcus pyogenes* for tonsillitis. The rate at which pathogenic bacteria are gaining resistance to these antibiotics is frightening. It is envisaged that *Phyllanthus amarus* aqueous leaf extract will be used to create treatments for tonsillitis brought on by *Streptococcus pyogenes*.

## RECOMMENDATIONS

Toxicity studies should also be done to determine safety. It's also recommended that similar studies should be done focusing on isolation of specific

phytochemicals of the plant and then establish their antibacterial activity against *Streptococcus pyogenes*.

## REFERENCES

1. Bartlett, A., Bola, S., Lt, S., & Williams, C. R. (2015). Acute tonsillitis and its complications : an overview, *101*(1), 69-73.
2. Bassetti, M., Manno, G., Collidà, A., Ferrando, A., Gatti, G., Ugolotti, E., ... Bassetti, D. (2000). Erythromycin Resistance in *Streptococcus pyogenes* in Italy, *6*(2), 180-183.
3. Cheruiyot, K. R., Olila, D., & Katereggia, J. (2009). In-vitro antibacterial activity of selected medicinal plants from Longisa region of Bomet district , Kenya, *9*(August).
4. DeWyer, A., Scheel, A., Webel, A. R., Longenecker, C. T., Kamarembo, J., Aliku, T., ... Beaton, A. (2020). Prevalence of group A  $\beta$ -hemolytic streptococcal throat carriage and prospective pilot surveillance of streptococcal sore throat in Ugandan school children. *International Journal of Infectious Diseases*, *93*(12), 245-251. <https://doi.org/10.1016/j.ijid.2020.01.013>
5. Haidara, A. W., Sidibé, Y., Samaké, D., Coulibaly, A., Touré, M. K., Coulibaly, B. B., ... Mohamed, A. A. (2019). Tonsillitis and Their Complications: Epidemiological, Clinical and Therapeutic Profiles. *International Journal of Otolaryngology and Head & Neck Surgery*, *08*(03), 98-105. <https://doi.org/10.4236/ijohns.2019.83011>
6. Hiremath, S., Kumar, H. D. V., Nandan, M., Mantesh, M., Shankarappa, K. S., Venkataravanappa, V., ... Reddy, C. N. L. (2021). In silico docking analysis revealed the potential of phytochemicals present in *Phyllanthus amarus* and *Andrographis paniculata* , used in Ayurveda medicine in inhibiting SARS - CoV - 2. *3 Biotech*, *11*(2), 1-18. <https://doi.org/10.1007/s13205-020-02578-7>
7. Ibrahim, D., Hong, L. S., & Kuppan, N. (2013). Antimicrobial activity of crude methanolic extract from *phyllanthus niruri*. *Natural Product Communications*, *8*(4), 493-496. <https://doi.org/10.1177/1934578x1300800422>
8. Ingle, K. P., Deshmukh, A. G., Padole, D. A., Mahendra, S., Moharil, M. P., & Khelurkar, V. C. (2017). Phytochemicals : Extraction methods , identification and detection of bioactive compounds from plant extracts, *6*(1), 32-36.
9. Kamatenesi, M. M., Acipa, A., & Oryem-Origa, H. (2011). Net Medicinal plants of Otwal and Ngai Sub Counties in Oyam District, Northern Uganda. *Journal of*

- Ethnobiology and Ethnomedicine*, 7(7),1-14.
10. Kurtz, B., Kurtz, M., & Roe, M. (2000). Importance of Inoculum Size and Sampling Effect in Rapid Antigen Detection for Diagnosis of Streptococcus pyogenes Pharyngitis, 38(1), 279-281.
  11. Lyskey, N. N., Lawrenson, R. A., & Sriskandan, S. (2011). New understandings in Streptococcus pyogenes, 1(24), 196-202. <https://doi.org/10.1097/QCO.0b013e3283458f7e>
  12. Ocan, M., Manabe, Y. C., Baluku, H., Atukwase, E., Ogwal-Okeng, J., & Obua, C. (2015). Prevalence and predictors of prior antibacterial use among patients presenting to hospitals in Northern Uganda. *BMC Pharmacology and Toxicology*, 16(1),1-8. <https://doi.org/10.1186/s40360-015-0027-8>
  13. Okiki, P. A., Egbebi, A., Akharaiyi, F. C., Adewole, E., & Asoso, S. O. (2022). Drug properties and antimicrobial evaluations of extracts from Phyllanthus amarus . *Journal of Microbiology & Experimentation*, 10(1), 10-16. <https://doi.org/10.15406/jmen.2022.10.00346>
  14. Owuama, C. I. (2017). Determination of minimum inhibitory concentration ( MIC ) and minimum bactericidal concentration (MBC) using a novel dilution tube method, 11(23), 977-980. <https://doi.org/10.5897/AJMR2017.8545>
  15. Patel, J. R., Tripathi, P., Sharma, V., Chauhan, N. S., & Dixit, V. K. (2011). Phyllanthus amarus: Ethnomedicinal uses, phytochemistry and pharmacology: A review. *Journal of Ethnopharmacology*, 138(2), 286-313. <https://doi.org/10.1016/j.jep.2011.09.040>
  16. Pathak, M., Singh, U. K., & Upadhyay, G. (2017). Antibacterial Activity of Phyllanthus Amarus Plant Extract Against Resistant Pathogenic Bacterial Strains: an Ethanomedicinal Plant. *Asian Journal of Science and Technology*, 8(September), 5672-5674.
  17. Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK. Phyllanthus amarus:ethnomedicinal uses, phytochemistry and pharmacology: a review. *J Ethnopharmacol*. 2011 Nov 18;138(2):286-313. doi: 10.1016/j.jep.2011.09.040. Epub 2011 Sep 29. PMID: 21982793.
  18. Bartlett, A., Bola, S., Lt, S., & Williams, C. R. (2015). Acute tonsillitis and its complications : an overview, 101(1), 69-73.
  19. Bartlett, A., Bola, S., Lt, S., & Williams, C. R. (2015). Acute tonsillitis and its complications : an overview, 101(1), 69-73.
  20. Bassetti, M., Manno, G., Collidà, A., Ferrando, A., Gatti, G., Ugolotti, E., ... Bassetti, D. (2000). Erythromycin Resistance in Streptococcus pyogenes in Italy, 6(2), 180-183.
  21. Cheruiyot, K. R., Olila, D., & Katereggga, J. (2009). In-vitro antibacterial activity of selected medicinal plants from Longisa region of Bomet district , Kenya, 9(August).
  22. DeWyer, A., Scheel, A., Webel, A. R., Longenecker, C. T., Kamaremba, J., Aliku, T., ... Beaton, A. (2020). Prevalence of group A  $\beta$ -hemolytic streptococcal throat carriage and prospective pilot surveillance of streptococcal sore throat in Ugandan school children. *International Journal of Infectious Diseases*, 93 (12), 245-251. <https://doi.org/10.1016/j.ijid.2020.01.013>
  23. Ocan, M., Manabe, Y. C., Baluku, H., Atukwase, E., Ogwal-Okeng, J., & Obua, C. (2015). Prevalence and predictors of prior antibacterial use among patients presenting to hospitals in Northern Uganda. *BMC Pharmacology and Toxicology*, 16(1),1-8. <https://doi.org/10.1186/s40360-015-0027-8>
  24. Okiki, P. A., Egbebi, A., Akharaiyi, F.

- C., Adewole, E., & Asoso, S. O. (2022). Drug properties and antimicrobial evaluations of extracts from *Phyllanthus amarus*. *Journal of Microbiology & Experimentation*, 10(1), 10-16. <https://doi.org/10.15406/jmen.2022.10.00346>
25. Owuama, C. I. (2017). Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method, 11(23), 977-980. <https://doi.org/10.5897/AJMR.2017.8545>
26. Patel, J. R., Tripathi, P., Sharma, V., Chauhan, N. S., & Dixit, V. K. (2011). *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology: A review. *Journal of Ethnopharmacology*, 138(2), 286-313. <https://doi.org/10.1016/j.jep.2011.09.040>
27. Pathak, M., Singh, U. K., & Upadhyay, G. (2017). Antibacterial Activity of *Phyllanthus Amarus* Plant Extract Against Resistant Pathogenic Bacterial Strains: an Ethnomedicinal Plant. *Asian Journal of Science and Technology*, 8(September), 5672-5674.
28. Bartlett, A., Bola, S., Lt, S., & Williams, C. R. (2015). Acute tonsillitis and its complications: an overview, 101(1), 69-73.
29. Bassetti, M., Manno, G., Collidà, A., Ferrando, A., Gatti, G., Ugolotti, E., ... Bassetti, D. (2000). Erythromycin Resistance in *Streptococcus pyogenes* in Italy, 6(2), 180-183.
30. Cheruiyot, K. R., Olila, D., & Katerega, J. (2009). In-vitro antibacterial activity of selected medicinal plants from Longisa region of Bomet district, Kenya, 9 (August).
31. DeWyer, A., Scheel, A., Webel, A. R., Longenecker, C. T., Kamaremba, J., Aliku, T., ... Beaton, A. (2020). Prevalence of group A  $\beta$ -hemolytic streptococcal throat carriage and prospective pilot surveillance of streptococcal sore throat in Ugandan school children. *International Journal of Infectious Diseases*, 93(12), 245-251. <https://doi.org/10.1016/j.ijid.2020.01.013>
32. Haidara, A. W., Sidibé, Y., Samaké, D., Coulibaly, A., Touré, M. K., Coulibaly, B. B., ... Mohamed, A. A. (2019). Tonsillitis and Their Complications: Epidemiological, Clinical and Therapeutic Profiles. *International Journal of Otolaryngology and Head & Neck Surgery*, 08(03), 98-105. <https://doi.org/10.4236/ijohns.2019.83011>
33. Hiremath, S., Kumar, H. D. V., Nandan, M., Mantesh, M., Shankarappa, K. S., Venkataravanappa, V., ... Reddy, C. N. L. (2021). In silico docking analysis revealed the potential of phytochemicals present in *Phyllanthus amarus* and *Andrographis paniculata*, used in Ayurveda medicine in inhibiting SARS - CoV - 2. *3 Biotech*, 11(2), 1-18. <https://doi.org/10.1007/s13205-020-02578-7>
34. Lynskey, N. N., Lawrenson, R. A., & Srisakandan, S. (2011). New understandings in *Streptococcus pyogenes*, 1(24), 196-202. <https://doi.org/10.1097/QCO.0b013e3283458f7e>
35. Ocan, M., Manabe, Y. C., Baluku, H., Atukwase, E., Ogwal-Okeng, J., & Obua, C. (2015). Prevalence and predictors of prior antibacterial use among patients presenting to hospitals in Northern Uganda. *BMC Pharmacology and Toxicology*, 16(1), 1-8. <https://doi.org/10.1186/s40360-015-0027-8>
36. Okiki, P. A., Egbebi, A., Akharaiyi, F. C., Adewole, E., & Asoso, S. O. (2022). Drug properties and antimicrobial evaluations of extracts from *Phyllanthus amarus*. *Journal of Microbiology & Experimentation*, 10(1), 10-16. <https://doi.org/10.15406/jmen.2022.10.00346>

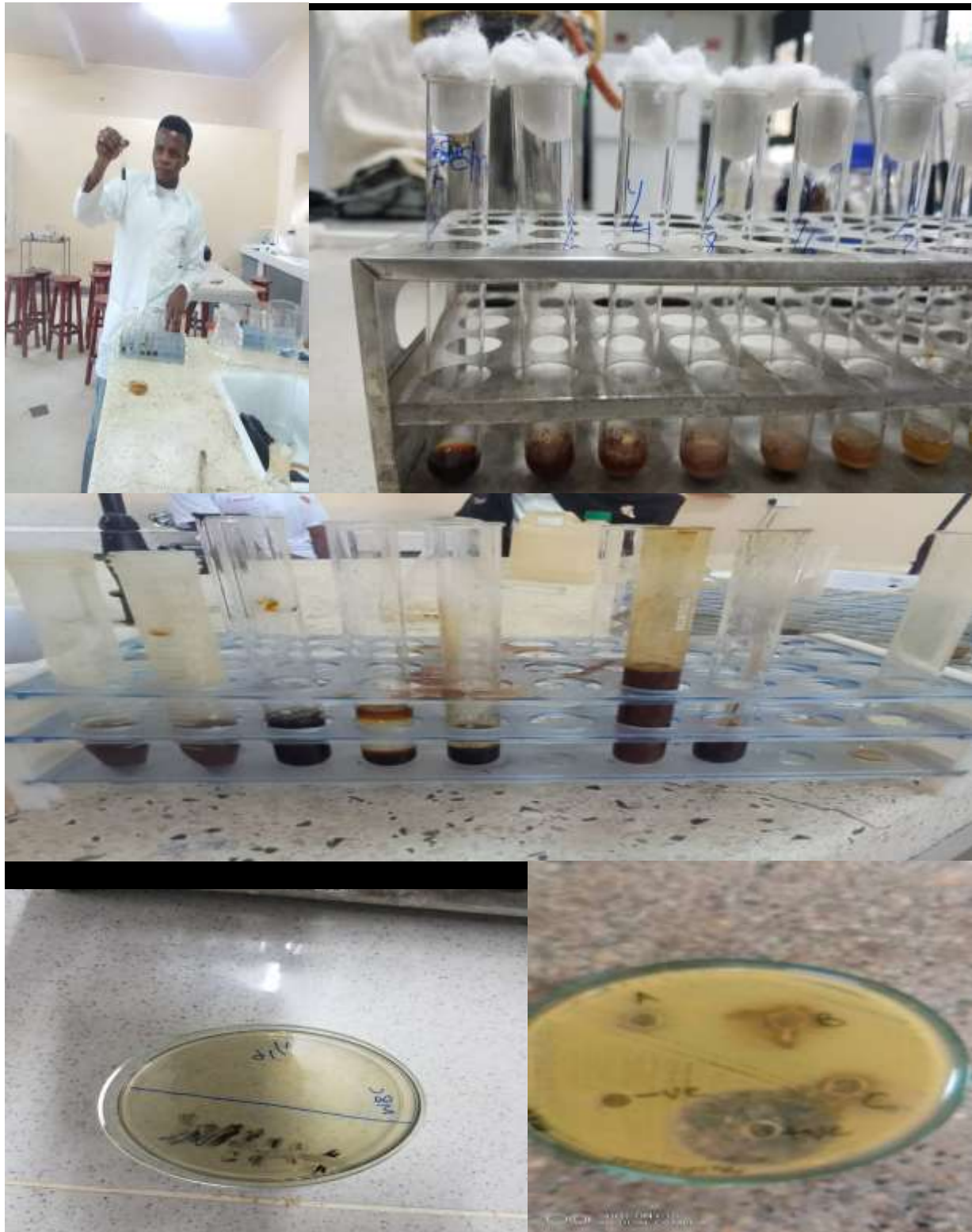
37. Owuama, C. I. (2017). Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method, *11*(23), 977-980. <https://doi.org/10.5897/AJMR.2017.8545>
38. Patel, J. R., Tripathi, P., Sharma, V., Chauhan, N. S., & Dixit, V. K. (2011). *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology: A review. *Journal of Ethnopharmacology*, *138*(2), 286-313. <https://doi.org/10.1016/j.jep.2011.09.040>
39. Pathak, M., Singh, U. K., & Upadhyay, G. (2017). Antibacterial Activity of *Phyllanthus amarus* Plant Extract Against Resistant Pathogenic Bacterial Strains: an Ethnomedicinal Plant. *Asian Journal of Science and Technology*, *8*(September), 5672-5674.
40. Asogwa, F. C., Ugwu, O. P. C., Alum, E. U., Egwu, C. O. and Edwin, N. (2015). Hygienic and sanitary assessment of street food vendors in selected towns of Enugu North District of Nigeria. *American-Eurasian Journal of Scientific Research*, *10*(1): 22-26.
41. Alum, E. U., Uti, D. E., Agah, V. M., Orji, O. U., Ezeani, N. N., Ugwu, O. P., Bawa, I., Omang, W. A. and Itodo, M. O. (2023). Physico-chemical and Bacteriological Analysis of Water used for Drinking and other Domestic Purposes in Amaozara Ozizza, Afikpo North, Ebonyi State, Nigeria. *Nigerian Journal of Biochemistry and Molecular Biology*, *2023*; *37*(1): 1-8.
42. Ministry of Health Uganda (MOH) (2016). National Guidelines for Management of Common Conditions.
43. Nicolle, L. E. (2008). Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. *Urol Clin North Am.*, *35*(1):1-12.
44. Longmore, Murray and others, (2014). 'Gastroenterology', *Oxford Handbook of Clinical Medicine*, 9edn, Oxford Medical Handbooks (Oxford, 2014; online edn, Oxford Academic, <https://doi.org/10.1093/med/9780199609628.003.0006>, accessed 24 Apr. 2023)
45. Hooton, T. M., Scholes, D., Stapleton, A. E, Roberts, P. L, winter, C., Gupta, K., Samadpour, M and Stamm, W. E. (2000). A prospective study of asymptomatic bacteriuria in sexually active young women. *N Engl J Med.*, *5*; *343*(14):992-7.
46. Gould, C. V., Umscheid, C. A, Agarwal, R. K, Kuntz, G. and Pegues, D. A (2010). Healthcare Infection Control Practices Advisory Committee. Guideline for prevention of catheter-associated urinary tract infections 2009. *Infect Control Hosp Epidemiol.*, *31*(4):319-26.
47. Perrotta, C., Aznar, M., Mejia, R., Albert, X. and Ng, C. W. (2008). Cochrane database of systematic reviews (Online) (2), 5131.
48. Feitosa, D. C., da Silva, M. G. and de Lima Parada, C. M. (2009). Accuracy of simple urine tests for diagnosis of urinary tract infections in low-risk pregnant women. *Rev Lat Am Enfermagem*, *17*(4):507-13.
49. Kolawole, A. S, Kolawole, O. M, Kandaki-Olukemi, Y. T, Babatunde, S. K and Durowade, K. A, (2009). Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. *International Journal of Medicine and Medical Sciences*, *1*(5): 163-167.
50. Turyasingura, E. (2023). Evaluation of the Urinary Retention in Matured Male Patients at Kampala International University Teaching Hospital in Bushenyi District Western Uganda. *INOSR Scientific Research* *9* (1), 50-58.
51. Gloria, N. Yamile, A. O. Agwu, E. (2022). Prevalence patterns of bacterial urinary tract infections among febrile children under-five years of age at Kampala



- International University Teaching Hospital. *IDOSR Journal of Biology, Chemistry and Pharmacy* 7 (1), 41-55.
52. Ilozue NM, UP Ikezu and PCU Okechukwu (2014). Antimicrobial and phytochemical screening of the seed extracts of *Persea americana* (avocado pear). *IOSR Journal of Pharmacy and Biological Sciences* 9 (2), 23-25.
53. Ibekwe Rita O and Ugwu Okechukwu P C Ikeyi Adachukwu P\*, Ogbonna Ann O (2013). Antimicrobial activity of *Xylopiya aethiopica* (UDA) on *Escherichia coli* and *Staphylococcus aureus* isolates from gastroenteritic patients. *International Journal of Life Sciences Biotechnology and Pharma Research*, 2 (3): 330-338.
54. Chukwuezi Fabian O. and P. Ugwu Okechukwu (2014). Antimicrobial effects of bitter kola (*Garcinia kola*) nut on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. *Journal of Dental and Medical Sciences* 13 (4), 29-32.
55. Chukwuezi Fabian O. and Ugwu Okechukwu P.C. (2013). Distribution of *Mycobacterium bacilli* in Onitsha Metropolis and its Relationship with HIV Infection. *Pharmanest An International Journal of Advances in Pharmaceutical Sciences*, 4(5): 902-906.
56. Onyeze RC, GT Onah, CL Onwukwe and OPC Ugwu (2013). Comparative effects of neem and lemongrass leaf extracts on *Salmonella* spp. *World Journal of Pharmaceutical Research* 2 (4), 1177-1185.



APPENDIX I: IMAGES OF LAB WORK



Epila Haron, Albert Nyanhoka Onchweri, Maniga Josephat, Tenywa Mercy, Jacqueline Njeri Muchiri and Ugwu Okechukwu Paul-Chima (2023). Evaluation of the anti-bacterial activity of aqueous leaf extract of *Phyllanthus amarus* on *Streptococcus pyogenes* for the treatment of tonsillitis. *IDOSR Journal of Biology, Chemistry and Pharmacy*, 8(2)47-62.<https://doi.org/10.59298/IDOSR/JBCP/23/10.125>.