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Original Article

ANTIBIOTIC ACTIVITY OF WHOLE LEAF EXTRACT OF *PHYLLANTHUS EMBLICA* AND *HELIANTHUS ANNUUS* AGAINST PENICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI.*

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ABSTRACT

Background:
More than 1 million sexually transmitted infections (STIs) are acquired every day worldwide. The study aims to assess the antibiotic activity of whole leaf extract of Phyllanthus embolic and Helianthus annuus against penicillin-resistant staphylococcus aureus and escherichia coli.

Methodology:
A laboratory-based experimental study was conducted for three months between November 2021 and January 2022. *S. aureus* and *E. coli* bacterial isolates from the bio-repository of the microbiology laboratory at the University of Kisubi (UniK) and the diagnostic laboratory at Our Lady of Consolata Kisubi hospital were retrieved and the selected plants were collected from the field; their fresh aqueous leaf extracts were prepared from which the respective antimicrobial susceptibility discs were made

Results:
S. aureus species were resistant to both *Phyllanthus Emblica* and *Helianthus Annuus*extracts. There was a higher average resistance to the *Helianthus Annuus* extract of 94.5% as compared to 90.0% of Phyllanthus *Emblica* extract. Both susceptibility and resistance profiles were considered depending on the antibiotic cefoxitin (Resistance <22≥ Susceptibility) measured in millimeters for zones of inhibition. *E. coli* species were resistant to both *Phyllanthus Emblica* and *Helianthus Annuus* extracts. There was a higher average resistance to the *Helianthus Annuus* extract of 89.3% as compared to 83.6% of Phyllanthus *Emblica* extract.
However, both susceptibility and resistance profiles were considered depending on the antibiotic Ampicillin (Resistance <14≥ Susceptibility) measured in millimeters for the zones of inhibition

Conclusion:
It was difficult to establish a bioactive-component profile and essential oils contributing towards antimicrobial activity from a specific plant species.

Recommendation:
Detailed qualitative and quantitative studies to distinguish antimicrobial agents in respective whole-leaf extracts need to be conducted and the establishment of therapeutic dosages

Keywords: *Antibiotic activity, Whole leaf extract, Phyllanthus emblica, Helianthus annuus, Penicillin-resistant staphylococcus aureus, Escherichia coli*
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BACKGROUND OF THE STUDY

More than 1 million sexually transmitted infections (STIs) are acquired every day worldwide (Rowley J *et al*., 2019). The most common sexually transmitted infections affecting the general population are Urinary tract infections (UTIs) of bacterial origin affecting 150 million people worldwide each year (Harding, Ronald., 1994), (O'brien *et al*., 2017). Although E. coli and K. pneumoniae are commensals, they have been mostly associated with urinary tract infections and sepsis. (Stanley *et al*., 2018) About 700,000 deaths per year worldwide are attributable to infections caused by six antimicrobial-resistant species, including E coli and K pneumonia (O'Neill,

2014)Antimicrobial The increasing rate of antimicrobial resistance (AMR) has resulted in a global threat and studies have shown that, by 2050, 10 million people will die every year due to (AMR) Kraker *et at*., (2016) this remains a serious public health threat despite decades of efforts to slow down the selection and transfer of resistance genes (Hoelzer *et al*.,2017). This has resulted in high rates of morbidity and high economic costs associated with recommended treatment regimens. A recent report by Kraker *et al.,* (2016) estimates that, by 2050, 10 million people will die every year due to antimicrobial resistance (AMR) until a global action to combat AMR is implemented. The World Health Organization (WHO) Global Action Plan on AMR for research to fill the knowledge gaps regarding infections caused by antimicrobial-resistant pathogens (WHO 2015) highly needs major attention.

Today, plant materials are playing a major role in primary health care as therapeutic in countries that are culturally bound and less developed for example studies done by Yusha*et al*., (2009) showed that the Carica papaya extracts that were tested for antibacterial activity against isolates of Escherichia coli, Klebsiella pneumoniae were able to inhibit growth at 1000µg/disc concentration. More studies done by Christina *et al*., (2017) showed that wild sunflowers had therapeutically relevant phytochemicals like tannins, cardiac glycosides, anthraquinones, flavonoids, alkaloids, anthocyanins, phlorotannins, carotenoids, steroids, saponins, phenolic and terpenoids. Evidence from Liasu, and Ayandele, (2008) showed that wild sunflowers had antimicrobial activity, active against both gram-negative bacteria and gram-positive, with activity against S. aureus, E. coli, and P. aeruginosa.

Research by Ördögh. (2010) has also shown that staphylococci were inhibited by the juices of Ribes and Rubus species with P. armeniaca and S. epidermidis being sensitive to the methanol extracts. This research shall provide knowledge about, the type of plant extract, and the lowest amount of plant extract in determining the susceptibility of selected strains of Escherichia coli and klebsiella peneuniae to selected plant extracts of bird gooseberry and wild sunflower obtained from central Uganda.

METHODOLOGY

Study design

This will be a laboratory-based experimental study conducted for three months between November 2021 and January 2022. *S. aureus* and *E. coli* bacterial isolates from the bio-repository of the microbiology laboratory at the University of Kisubi (UniK) and the diagnostic laboratory at Our Lady of Consolata Kisubi hospital were retrieved and the selected plants were collected from the field; their fresh aqueous leaf extracts were prepared from which the respective antimicrobial susceptibility discs were made. Determining and comparing the antibacterial activity of the whole leaf extracts of the mentioned plants against penicillin-resistant *S. aureus* and *E. coli*then followed.

Study site

Site for laboratory experimentation

All the relevant laboratory experimentation will be carried out at the microbiology laboratory of the faculty of health sciences at the University of Kisubi. The laboratory is located on the ground floor of the science block. This was done following formal permission from the management board of the faculty of health sciences at the University of Kisubi.

Sites for collection of the bacterial isolates (S. aureus and E. coli)

The two bacterial isolates i.e., *S. aureus and E. coli* will be retrieved from the bio-repository of two sites i.e.

(i) The bio-repository of the faculty of health sciences at the University of Kisubi and

(ii) The bio-repository at Our Lady of Consolata Kisubi Hospital.

This was done following permission from the management at both sites.

Site for the collection of selected plant species

Plant samples (*Phyllanthus emblica* and *Helianthus annuus)* will be collected during afternoon hours when phytochemicals are at maximum concentrations from UniK plantations (From Fr Angello Ssemugooma's Botanical Garden). These samples then will be placed in a sterile polythene bag, and properly labeled using initials given by the researcher. The collected samples will then be transported to the UniK microbiology lab at the Science Block. To avoid contamination of the samples, the plant samples (*Phyllanthus emblica* and *Helianthus annuus)* were washed with running tap water and then rinsed properly with sterile distilled water.

Inclusion and Exclusion criteria

Inclusion Criteria

- Fresh healthy plant leaves.
- Penicillin-resistant *S. aureus* and *E. coli* bacterial isolates.

Exclusion Criteria

- Non-viable isolates which are susceptible to penicillin.
- Dried and damaged plant leaves.

Study organisms

Penicillin-resistant isolates of S. aureus and E. coli studied from UniK and Kisubi Hospital laboratory biorepository will be obtained and subjected to the different extracts for study.

Staphylococcus Aureus

S. aureus is a facultative gram-positive and cocci-shaped bacterium forming clusters on media. It's catalase, coagulase-positive, and a mannitol fermenter. It's a normal flora on healthy skin, however a causative agent of multiple human infections implicated in both community-acquired and hospital-acquired infections that are of clinical significance. Resistant strains of this species have the *mec gene that* encodes the protein PBP-2a (Penicillin Binding Protein 2a) which is essential for the enzyme that catalyzes the production of peptidoglycan in the bacterial cell wall. This PBP-2a has a lower affinity of binding to *beta-lactams*compared to other PBPs. Therefore, *S. aureus* continues to synthesize bacterial cell walls in the presence of many antibiotics (Taylor and Unakal., 2021).

Escherichia Coli

*E. coli*is a gram-negative rod, motile in some species, a facultative anaerobe forming pink colonies on MacConkey agar. The organism is indole positive, citrate, and urea negative respectively, and forms gas but H2S is not formed. *E. coli* normally resides in the lower intestines of warm-blooded mammals and thus its versatility in the microbiota as a commensal. Previously it was susceptible to various antibiotics, but recently, *E. coli* has developed a great capacity to accumulate resistant genes observed in horizontal gene transfers with genes that encode for extended-spectrum *beta-lactamases, rRNA methylases, plasmid-mediated quinolone resistance genes*; and sharing of multi-resistance plasmids among other mobile genetic elements within biofilms, play a great role in the dissemination of resistance. In addition to the above, the use of antibiotics in both veterinary and Human medicine at varying concentrations makes zoonoses out brakes having *E. coli* of animal origin among humans and treatment becomes more difficult due to earlier exposures.

Plant species selected for whole-leaf extraction

In folk medicine, medicinal plants are precious gifts of nature, which may serve as a source of food and medicine to humans. *Phyllanthus emblica* L has held a unique position in the Indian (Ayurvedic), Turkish, Unani, and Tibetan medicinal systems for centuries. Its nutritional, therapeutic, and healing potentials have made it a valid research option for the development of novel drug formulations with few side effects. The presence of vitamin C, alkaloids, ellagitannins, gallic acid, emblicanin A and emblicanin B, flavonoids (especially rutin and quercetin), and a variety of biological molecules, makes *P. emblica*, a valued medicinal plant (Ahmad et al., 2021). It is used in ethnomedicine for treating several disease conditions which include heart disease, bronchial, laryngeal, and pulmonary affections, coughs and colds, and whooping cough (Tasneem et al., 2015).

*H. annuus*is pharmacologically studied for various activities including gantiinflammatory, anti-oxidant, antitumor, antiasthmatic, antigen, antipyretic, astringent, antihypoglycemic effect, antifungal activities, cathartic, diuretic, stimulant, vermifuge, vulnerary purposes, and antimicrobial activities (Tasneem et al., 2015). The plant *H. annuus*, was used as food and medicine worldwide. It was cultivated basically for its seeds, which give the world's second most important source of edible oil. Phytochemical analysis showed that *Helianthus annuus* contained carbohydrates, phenolics, flavonoids, tannins, alkaloids, saponins, phytosterols, and steroids among others (Brobbey et al., 2020).

Sample size determination

The number of samples dealt with during this surveillance will be determined using the "central limit theory." This theory considers any sample size above 30% to be a sufficient representation of the population.

In this study, the theory will work best as per the research design considered (http://sphweb.bumc.bu.edu/otlt/MPHModules/QuantCore/PH717_Probability/PH717_Probability8.html). From this; a total outcome of any size above 30 (if N=100) organisms is to be considered. In this study, a sample size of 30 sample samples was considered.

Materials

The materials to be used in the study include; pipettes, agar plates, an incubator, a bio bag, a refrigerator, methanol, distilled water, markers and labeling pens/pencils, samples, a research book, a hot air oven, an autoclave, gloves, conical flasks, motor and pestle, wire loops, culture media (Nutrient agar, peptone water), MacFarland, fire lamp and a personal computer.

Bacterial retrieval and Identification

Stocked organisms are to be obtained from refrigerator and/or freezer units (biorepository units) at UniK

Microbiology laboratory and Our Lady of Consolata Kisubi Hospital diagnostic laboratory.

Media Preparation

Nutrient agar and Peptone will be prepared under the independent manufacturer's manuals (Mast Group Limited, Merseyside UK).

Preparation of standard culture inoculum of test organisms

Isolated colonies shall be inoculated in 2ml normal saline until the growth concentration is equivalent to Mac-Farland (0.5%) recommended by WHO.

Antibacterial assay

Agar well diffusion assay shall be carried out to determine the antibacterial ability and activity of whole leaf extracts as follows
300mls Nutrient agar will be prepared following the manufacturer's instructions (Mast Group Limited, Merseyside UK).

a) and then autoclaved at 1210C for 15min in a conical flash
b) Sterile culture plates are on which the media will be dispensed each @25mls and left to set.
c) They were incubated at 370C for 24 hours sterility testing.
d) MacFarland standard inoculum was inoculated for each organism and spread evenly using sterile swabs on sterile media.

Obtaining of extract

Specimens are to be crushed using a motor and pestle to obtain the aqueous extract that is to be utilized in the making of antimicrobial disks.

Antimicrobial susceptibility test discs

Five filter papers attached will be punched using a cleaned punching machine to obtain discs, these shall later be submerged in the whole leaf extracts accordingly. These shall be placed on the inoculated Nutrient agar.
Inhibition shall be determined by the presence of a zone of clearance (no bacterial growth) by the whole leaf extract.

Quality assurance and quality control

Data quality will be ensured through standardized data collection materials
All media to be used will have to be subjected to sterility testing

Data collection and analysis

The raw data above will be collected in my research book, then summarized, and arranged with the aid of the Microsoft Excel computer application. The summarized and arranged data will then be analyzed with the same application to generate tables and/or graphs that will later be presented. A personal password-protected computer only accessible to the researcher will be utilized for this purpose.

Antimicrobial susceptibility test discs

Five filter papers attached will be punched using a cleaned punching machine to obtain discs, these shall later be submerged in the whole leaf extracts accordingly. These shall be placed on the inoculated Nutrient agar.
Inhibition shall be determined by the presence of a zone of clearance (no bacterial growth) by the whole leaf extract.

RESULTS

This study aimed to investigate the antibiotic activity of whole leaf extract of *Phyllanthus Emblica* and *Helianthus Annuus* against penicillin-resistant *Staphylococcus Aureus* and *Escherichia Coli.*
Aqueous whole leaf extracts (*Phyllanthus Emblica* and *Helianthus Annuus*) profiles against penicillin-resistant S. Aureus

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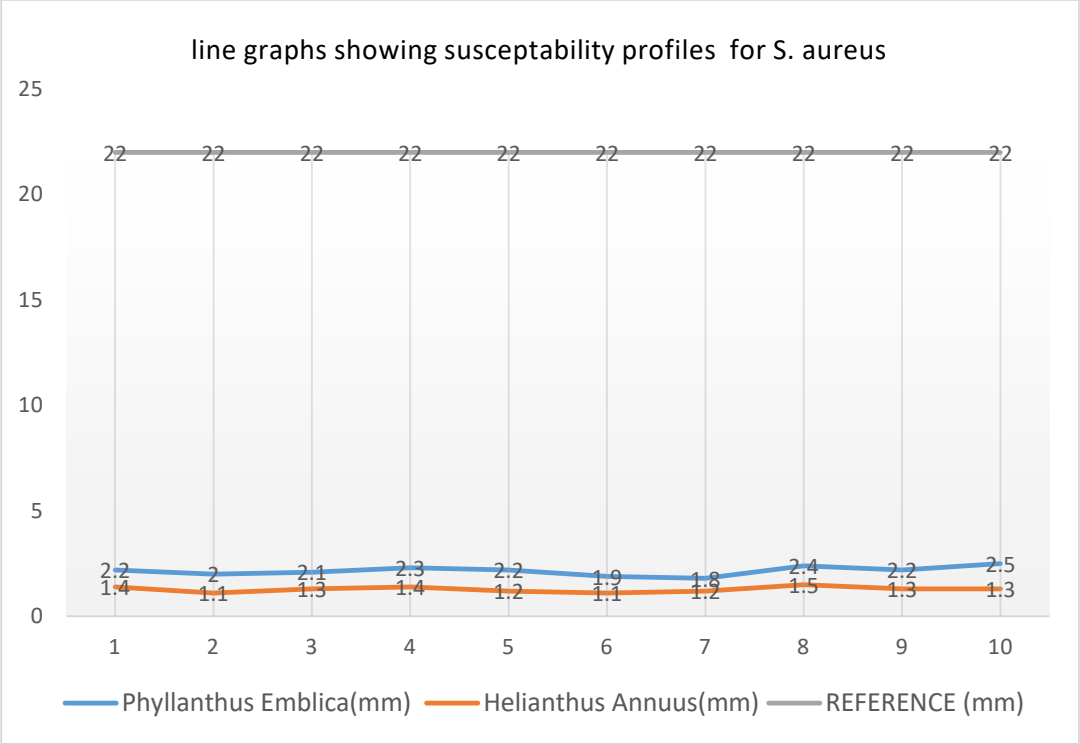
Graph 1: Showing antibiotic profiles against penicillin-resistant S. aureus.



Key; reference inhibition zone, organisms below are resistant at 22mm

Note: *The zones of inhibition profiles (mm) were interpreted by comparing them to the EUCAST standard charts.*

GRAPH 1: (n=10) The *S. aureus* species resisted *Phyllanthus Emblica* and *Helianthus Annuus* extracts. There was a higher average resistance to the *Helianthus Annuus* extract of 94.5% as compared to 90.0% of *Phyllanthus Emblica* extract.

However, susceptibility and resistance profiles were considered depending on the antibiotic cefoxitin (Resistance <22≥ Susceptibility) measured in millimeters for zones of inhibition. Therefore, graph 1 shows the details of each sample ID, depending on its susceptibility and resistance distance. Some samples are more resistant compared to others and vice versa.

Aqueous whole leaf extracts (*Phyllanthus Emblica* and *Helianthus Annuus*) profiles against penicillin-resistant E. coli

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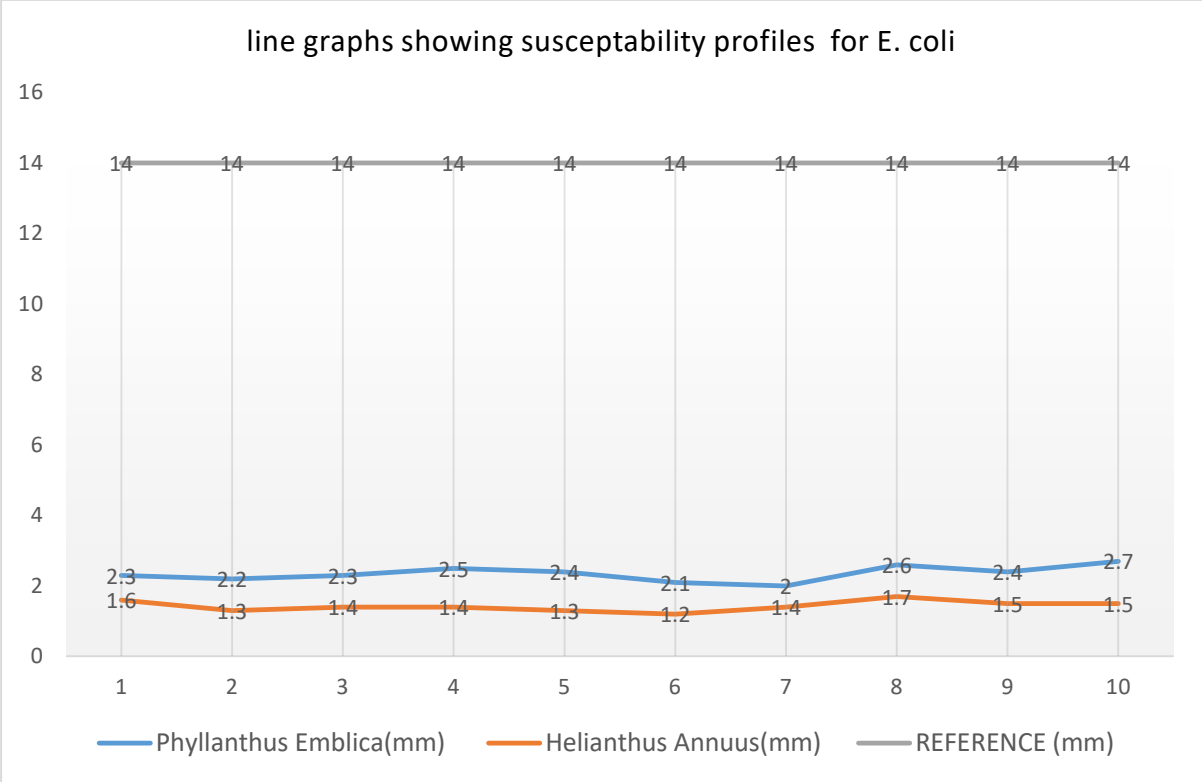
Graph 2: Showing antibiotic profiles against penicillin-resistant E. coli.



Key; reference inhibition zone, organisms below are resistant at 14mm

Note: *the interpretation of the zones of inhibition profiles (mm) was made by comparing them to the EUCAST standard charts.*

From Graph 2; (n=10) the *E. coli* species were resistant to both *Phyllanthus Emblica* and *Helianthus Annuus* extracts. There was a higher average resistance to the *Helianthus Annuus* extract of 89.3% as compared to 83.6% of Phyllanthus *Emblica* extract.

However, both susceptibility and resistance profiles were considered depending on the antibiotic Ampicillin (Resistance <14≥ Susceptibility) measured in millimeters for the zones of inhibition. Therefore, the graph shows the details of each sample ID, depending on its susceptibility and resistance pattern. Some samples are more resistant compared to others and vice versa.

Hypotheses on antibiotic profiles of aqueous extracts (*Phyllanthus Emblica and Helianthus Annuus*)

Phyllanthus Emblica whole leaf aqueous extract statistically supports the null hypothesis; it has an 11% response to penicillin-resistant *S. Aureus* and a 17% response to resistant *E. Coli* respectively. Thus, organisms could even grow on the leaf extract disc.

Helianthus Annuus whole leaf aqueous extract statistically supported the null hypothesis with an 08% and 12% response against both penicillin-resistant *S. Aureus* and *E. Coli* respectively.

DISCUSSION OF STUDY FINDINGS

In this study, the antibiotic profiles of Penicillin-resistant *S. Aureus* and *E. Coli* against whole-leaf aqueous extracts of *Phyllanthus Emblica* and *Helianthus Annuus* were determined. Out of these 20 samples, 10 were *S. Aureus* and the other 10 were *E. Coli.* From the study findings (n=10 for each organism) the organisms were highly resistant to both *Phyllanthus Emblica and Helianthus Annuus* aqueous extracts.

Findings showed a close to zero response on average of the plant (*Phyllanthus Emblica* and *Helianthus Annuus)* aqueous extract to penicillin-resistant *S.aureua* and *E. coli*. A study by Brobbey et al (2020) concluded that both plants had antibiotic properties but didn't document the response toward resistant bacteria. In addition, different studies. However, results from this study showed a 94.5% and 90.0%

resistance pattern to *Helianthus Annuus* and *Phyllanthus Emblica* the whole-leaf aqueous extracts respectively. This therefore indicates that the difference in the study findings can be attributed to the consideration of penicillin-resistant organisms only yet other studies didn't consider organisms' resistance patterns.

It should also be noted that *S. aureus* had a higher resistance pattern to both extracts that at certain incidences during the study, zones of inhibition were read at 1.9mm in some species yet the reference range was at 22mm. Although more studies are needed, we concluded that the aqueous plant extract (*Phyllanthus Emblica)* might have a component inhibiting other bacterial species.

In this study, antimicrobial activity is recorded at 5.5% highlighting the impact of resistance mechanisms used by these bacterial species. Mahizam et al (2019) concluded that phytosterols and flavonoids were highly responsible for the plant's (*H. annuus)* antibiotic pattern in the different bacterial species. However, no study had been conducted considering isolated resistant species on this plant, but in this study, bacterial isolates exhibited higher resistance as compared to other studies. Therefore, statistically, both of the two organisms were resistant to these plant extracts. In other studies, Ferraz et al (2020), and Aslam et al (2018) a susceptibility pattern of non-penicillin-resistant organisms especially gram-positive bacteria, showed an antimicrobial activity of over 90% on average for both plant species (*Phyllanthus Emblica* and *Helianthus Annuus)* respectively.

CONCLUSION

It was difficult to establish a bioactive-component profile and essential oils contributing towards antimicrobial activity from a specific plant species.

RECOMMENDATION

Detailed qualitative and quantitative studies to distinguish antimicrobial agents in respective whole leaf extracts need to be conducted and establishment of therapeutic dosages.
Knowing the specific phytochemical profile of a specific plant part (leaf) instigates further research into *combination therapies* between a known antimicrobial agent (Penicillin, Ampicillin, Cefoxitin, etc.) with natural compounds and other drugs to recover the loss of function for the existing antimicrobial agents. This therefore potentiates the action of existing drugs and appreciates the multi-targeted pharmacokinetic effect of novel therapies in the clearance/destruction of existing AMR mechanisms among the different pathogenic bacteria. In this study, it was observed that earlier resistance was recorded on non-resistant species previously, however, our results showed little antimicrobial activity by the plant (*Phyllanthus Emblica* and *Helianthus Annuus)* aqueous extracts. Thus, according to study findings, the little resistance could be boosted using combination therapies with specific antibiotics to have a significant effect against different resistant pathogenic bacteria. In addition, different phytochemicals can be purified and manufactured on a large scale and then used as antimicrobial agents at recommended concentrations, especially for gram-negative pathogenic bacterial species.

LIST OF ABBREVIATIONS

%: Percent
AMR: Anti-Microbial Resistance
E. coli: *Escherichia coli*
EUCAST: European Union Charts for Antibiotic Susceptibility Testing
Lab: Laboratory
NA: Nutrient Agar
S. aureus: *Staphylococcus aureus*
STIs: Sexually Transmitted Infections
UNIK: University of Kisubi
UTI: Urinary Tract Infection
WHO: World Health Organization

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CONFLICT OF INTEREST

The author did not declare any conflict of interest

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Fred Parmu is a student of Bachelor of Biomedical Laboratory Technology at the University of Kisubi.
Fortunate Lujjimbirwa is a lecturer at the faculty of health sciences of the University of Kisubi.

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