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Lusweti Kituyi

Department of Chemistry and Biochemistry, University of Eldoret, P.O Box 1125-31000 Eldoret, Kenya

Samson M. Lutta

Department of Chemistry and Biochemistry, University of Eldoret, P.O Box 1125-31000 Eldoret, Kenya

Steve Barasa

Department of Chemistry and Biochemistry, University of Eldoret, P.O Box 1125-31000 Eldoret, Kenya

Antimicrobial activity of *Artemisia annua* L and *Aloe barbadense* miller plant extracts against *Staphylococcus aureus*

Lusweti Kituyi, Samson M. Lutta, Steve Barasa

ABSTRACT

Artemisia annua L. and *Aloe barbadense* miller are medicinal plants applied in treatment of various diseases. *Staphylococcus aureus* is a gram-negative bacterium found commonly on the skin and in the environment. Phytochemical are plants secondary metabolites with promising medicinal properties as remedy to limitations associated with the conventional drugs. A study was conducted to investigate the anti-microbial properties of bioactive components from natural leave extracts of *Artemisia annua* L. and *Aloe barbadensis* miller obtained from methanolic as extractant against *Staphylococcus aureus*. The phytochemical analysis revealed the leave extracts contained most plant secondary metabolites. The biochemical components from both plants were established to inhibit the growth of *Staphylococcus aureus*. *Artemisia annua* L had an inhibition zone of 20.67 mm while *Aloe barbadensis* miller had 10.33 mm against *Staphylococcus aureus*. The anti-microbial activity of the combined leaves extracts displayed significant high levels of synergetic properties with an inhibition zone of 23.67 mm. Gentamicin which served as standard for the assay had an inhibition zone of 27.67 mm. The study hence showed *Artemisia annua* L and *Aloe barbadensis* miller leaves bioactive components can be applied as an effective alternative medicine in management of *Staphylococcus aureus*.

Keywords: Synergism, Pharmacognosy, Pathogens, Microbes, Polytherapy, Alkaloids.

INTRODUCTION

Anti-microbial drug resistance associated with use of conventional medicine is a major challenge in the medical sector ^[1]. Aloe vera (*Aloe barbadensis miller*) has been applied since time in memorial as drug component in management of various animal diseases ^[2]. *Aloe barbadensis* is a natural plant that has been studied and administered on multiple diseases as oral or topical medicine ^[3]. Research have not only confirmed but also depicted the various pharmacological mechanisms related to *Aloe barbadensis miller* as an effective medicinal plant ^[4].

Artemisia annua L contains sesquiterpene lactone as bioactive component and has been used as an effective anti-malaria medicament ^[5]. *Artemisia annua* L natural products with therapeutic properties have efficaciously been explored for anti-microbial activities ^[6]. Some recent studies have demonstrated *Artemisinin annua* L inhibit microbes by degrading proteins and proteasome altering their metabolism ^[7]. The combination of various medicine plants extracts can be a remedy for microbial drug resistance ^[8].

Staphylococcus aureus remains one of the most significant microbes in the community globally and a major challenge in health sector ^[9]. The microbe's dominant habitat is the skin where they multiply enhancing their pathogenicity ^[10]. *Staphylococcus aureus* pathogen is linked to numerous human diseases once it ingested into the internal tissues ^[11]. *Staphylococcus aureus* is one of the microbes that has contributed to the upsurge of anti-microbial resistances ^[12].

Research on phytomedicine is going on with aim of utilizing plant extracts biochemical components as a solution to anti-microbial drug resistance and their side effects ^[13]. Phytochemicals possess pharmacological bioactivities that can enhance drug efficacy against several ailments ^[14]. Alkaloid a plant secondary metabolite has been found to exhibit phytopharmacological features that can elevate the activities of anti-microbial agents ^[15]. Thus, a study was undertaken to determine the possibility of synergism interactions due to polytherapy involving methanolic extract of *Artemisia annua* L and *Aloe barbadensis miller* against *Staphylococcus aureus*.

MATERIALS AND METHODS

Sampling and Experimental site

Correspondence:

Prof. John Lusweti Kituyi

Department of Chemistry and Biochemistry, University of Eldoret, P.O Box 1125-31000 Eldoret, Kenya

Email: joluki@yahoo.com

The *Artemisia annua L* plant was obtained from Bungoma county and *Aloe barbadensis miller* from Uasin Gishu county. Both plant specimens were identified at the University of Eldoret department of biological sciences herbarium section. This research was conducted in University of Eldoret, Kenya a region with an altitude of 2090 m, latitude of 00° 55' North and longitude of 34° 50' East. The Materials used for analysis were sterilized to ensure aseptic conditions are maintained during the experiments.

Preparation of extracts

This was done as described by [16] with few modifications. *Aloe barbadensis miller* gel was extracted from fresh leaves placed in sterilized bottle and refrigerated. The leaves of the plant specimens were naturally dried, grind, and weighed .10 g of dried leaves powder of each of the plants were separately soaked in 100 ml of methanol, macerated and left for 3 days for extraction process to complete. The extract solutions were filtered under vacuum then concentrated using rotor evaporator and air dry for few minutes to ensure the extracts were completely dried.

1mg/ml solution of *Artemisinin annua L* plant extract was prepared by mixing 100 mg of extracts in 100 ml sterilized distilled water and transferred in a sterilized amber bottle. 1mg/ml solution of *Aloe barbadensis miller* was prepared by dissolving 50 mg of the dried extract and the gel in 100 ml sterilized distilled water. Extract's test solution was prepared by mixing 50ml each of 1mg/ml *Artemisinin annua L* and *Aloe barbadensis miller* of the stock solutions. The prepared solutions were transferred in brown amber bottle and stored in a refrigerator as extract test solution.

Phytochemical Test

The test was done as illustrated by [17] using of mixture *Artemisinin annua L* and *Aloe barbadensis miller* extracts as test solution. Anthraquinones analysis was conducted by adding few drops of concentrated HCL in 2 cm³ of the extracts test solution. This was followed by heating in a water bath for few minutes, filtering and cooling. 2cm³ chloroform and 3 cm³ of 6M NH₄OH were then added to the filtrate, which was observed for pink-rose colour as confirmation for the presence anthraquinones. Glycosides analysis was performed by mixing 2 cm³ each of the extract's solution, chloroform and acetic acid, the appearance of bluish green colour indicated presence glycosides.

The phytochemical screening for alkaloids was accomplished by dissolving 3 cm³ of the extract's solution in 3 cm³ of concentrated hydrochloric acid followed by heating in water bath. The mixture was then transferred into a test-tube containing 3 cm³ each of Mayer's reagent and Wagner's reagents, the generation of suspension in the solutions revealed the presence of alkaloids. Flavonoids assay was carried out by mixing 2 cm³ of extracts solution with 2 cm³ of 10% Pb(C₂H₃O₂)₂ and the formation of a yellowish solid deposits was taken as an indicator for flavonoids.

Steroids analysis was performed by mixing 2 cm³ each of the extract's solution, chloroform and concentrated H₂SO₄. The presence of red colouration in the aqueous layer was taken as confirmatory test. The test for tannins was done by mixing 2 cm³ of the extracts, 3 cm³ distilled water and few drops of 0.3 M FeCl₃, the appearance of green colour was taken as positive. Saponins were determined by mixing 5 cm³ of extracts solution with equal volume of distilled water followed

gently warming where the appearance of foam indicated the presence of saponins in the analyte.

Terpenoids were determined by transferring 2 cm³ each of extracts and chloroform in a test tube, the solution was then evaporated to dryness. This was followed by addition of 2 cm³ concentrated H₂SO₄ and warming of mixture in water bath for two minutes, where the formation of greyish colour indicated presence of terpenoids. All the analyses for phytochemicals in the extracts were performed concurrently with a blank which contained only sterilized distilled water that was subjected to entire phytochemical tests but gave negative results.

Antimicrobial activity of treatments against *Staphylococcus aureus*

This was done using disc diffusion techniques as explained by [18] with some minor modifications. The materials and reagents were sterilized in an autoclave at 121 °C. The glass wares used had been previously cleaned with disinfectant, rinse, dried and sterilized in an oven at 100 °C. The surrounding environs including the benches were disinfected using absolute ethanol to ensure the aseptic conditions are maintained while the microbial inoculation was performed inside a laminar flow hood in a consistence environ saturated with ethanolic aerosol.

Nutrient agar was prepared, sterilized in an autoclave, cooled and inoculated with *Staphylococcus aureus* in a sterilized petri dish (diameter 9 cm). Disc filter paper each were impregnated with 50 µl of the extracts test solution and applied on the surface of the plate to inhibit bacteria growth. They were then transferred in an incubator set at temperature of 37°C for 24 hrs. and the inhibition zones determined. The same procedure was repeated for 1mg/ml of gentamicin antibiotic which served as positive control and the negative control which contained only sterilized distilled water as test solution for the microbial analysis. The microbial assay tests were done in triplicate

Statistical Analysis

The statistical analysis was performed using R statistical software where one-way ANOVA was used to evaluate the correlation between the treatment solutions and the inhibition zones for *staphylococcus aureus*. Tukey's test was applied in assessing variation between means from difference groups. During the analysis results the value p < 0.05 was considered to be significant statistical.

RESULTS

Table 1: Qualitative Phytochemical tests for *Artemisinin annua L* and *Aloe barbadensis miller*

| Phytochemicals | AL-AM |
|----------------|-------|
| Anthraquinones | + |
| Glycosides | + |
| Alkaloids | + |
| Flavonoids | + |
| Alkaloids | + |
| Steroids | - |
| Tannins | + |
| Saponins | + |
| Terpenoid | + |

AL-AM: Methanolic extracts of A. annua L and A. barbadensis miller; + Present - Absent

Antimicrobial activity of treatments against *Staphylococcus aureus*

Table 2: Zone of inhibition

| Extract | Zone of inhibition (mm) | | | Mean |
|---|-------------------------|-------|-------|-------|
| <i>Artemisia annua L</i> | 22.00 | 20.00 | 20.00 | 20.67 |
| <i>Aloe barbadensis miller</i> | 11.00 | 10.00 | 10.00 | 10.33 |
| <i>Artemisia annua L</i> + <i>Aloe barbadensis miller</i> | 25.00 | 23.00 | 23.00 | 23.67 |
| Positive control | 29.00 | 28.00 | 26.00 | 27.67 |
| Negative control | 00.00 | 00.00 | 00.00 | 00.00 |

Positive control consist of 1mg/ml gentamicin and the negative control was sterilized distilled water used for preparation of treatment solutions. Tukey's test was applied in determining significant variance between the means.

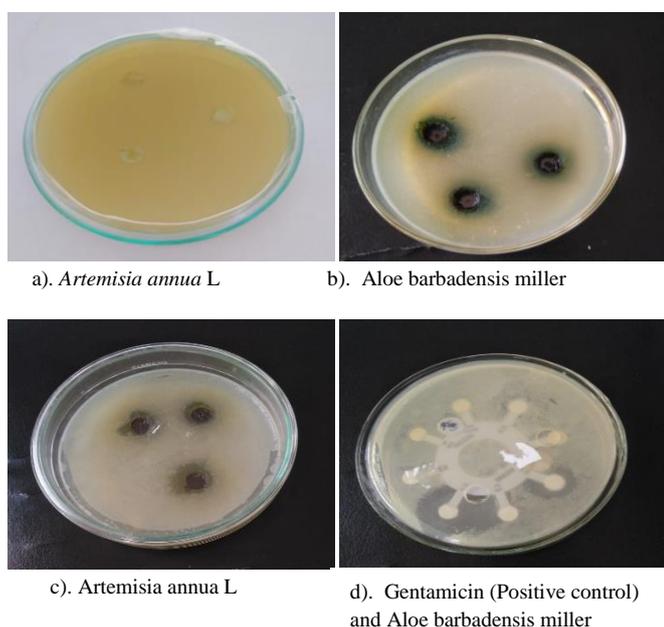


Figure 1: The mean ± SEM inhibition zones designated at 95% confidence limit for a). *Artemisia annua L* = 20.67mm b). *Aloe barbadensis miller* =10.33mm c). *Artemisia annua L* + *Aloe barbadensis miller* = 23.67mm and d). Gentamicin= 26.67mm.

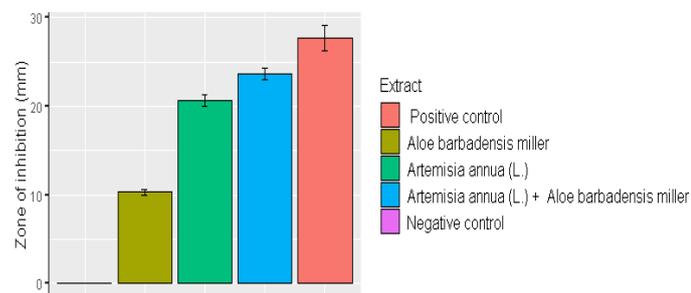


Figure 2: Variations in inhibition zones with treatments on *Staphylococcus aureus*, the bar represents mean ± SEM; n = 5 and p < 0.05 (One -Way ANOVA with turkey test for group means). The Positive control = 1mg/ml gentamicin and Negative control used sterilized distill water.

DISCUSSION

The finding from phytochemical analysis revealed the extracts contains most of plant secondary metabolites including flavonoids, saponins among others apart from steroid which might have been due

to the polarity of the extractant (Table 1). Phytochemicals are plant secondary biomolecules nutraceuticals that have been proven to provide defense against human diseases using various mechanisms [19]. Research on biochemical constituents of plant extracts have shown they possess functional groups that can inhibit the development and activities of microbial pathogen [20]. The outcomes from this study can also be interrelated with research that revealed saponin, alkaloid and terpenoid were present in extracts of plants *Moringa oleifera* that exhibited substantial anti- bacterial phytopharmacological activities [21].

The inhibition zones result of the treatment solutions significantly varied (Figure 1). *Aloe barbadensis miller* had the lowest with mean of 10.33 mm followed by *Artemisia annua L* with an inhibition zone of 20.67 mm. The combine extracts *Artemisia annua L* + *Aloe barbadensis miller* had a significant higher inhibition zone of 23.67mm compared with each of the plant extracts separately. This concurs with some pharmacognosy study that disclosed combination of different medicinal plants can be used for preventive measures and to increase therapeutic efficacy of drugs [22]. Gentamicin which serves as standard for positive control during the assay had highest inhibition zones of 27.67mm while negative control recorded no zone inhibition.

Plant extracts contain various medicine components which when combined together improve their potency and efficacy against diseases [23]. The synergetic properties of the two extracts can also be attributed to increase in bioavailability of saponins which interacts with lipid A in the bacterial cell wall and enhance permeability of medicinal components into bacterial cell [24]. This can also be ascribed to flavonoids which act as anti-microbial agents by destroying the cytoplasmic membrane resulting to suppress metabolic energy which impedes biosynthesis pathway of nucleic acid in bacterial cell [25].

CONCLUSION

Artemisia annua L and *Aloe barbadensis* have phytochemicals that possess pharmacological properties. The two extracts combined also exhibited synergism that significantly increased the inhibition of microbial activities due to *Staphylococcus aureus*. Thus, the two herbals combined have the aptitude to develop a more effective broad-spectrum herbal formulation not only as anti-*Staphylococci* but as an ingredient with multi- therapeutic agent having superior potency and efficacy.

Conflict of interest

The authors confirm they is none.

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ORCID ID

Lusweti Kituyi: <https://orcid.org/0009-0003-7247-992X>
 Samson M. Lutta: <https://orcid.org/0009-0006-9956-9441>
 Steve Barasa: <https://orcid.org/0000-0003-1116-1523>

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