

Antimicrobial Potency of Methanolic Leaf Extracts from Selected Medicinal Plants against *Staphylococcus aureus*

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Rec date: Dec 24, 2015; Acc date: Feb 13, 2016; Pub date: Feb 18, 2016

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Abstract

The main aim of the study was to test for the antimicrobial potency of *Aloe secundiflora*, *Bulbine frutescens*, *Tagetes minuta* and *Vernonia lasiopus* against *Staphylococcus aureus*. All the plants showed a pronounced antimicrobial activity against *Staphylococcus aureus* with *Tagetes minuta* being the most active at low concentrations (MIC 8.9 mg/ml; MBC 10.0 mg/ml) whereas *Vernonia lasiopus* showing less activity (MIC 12.2 mg/ml; MBC 14.2 mg/ml). The efficacy test was carried out using the disc diffusion method. The standard antibiotics used were ciprofloxacin (5 µg/ml) and vancomycin (3 µg/ml) showed significant antimicrobial activity by producing zones of inhibition of 22 mm and 25 mm respectively. Dimethyl sulphoxide and distilled water were used as negative control. The extracts from the plants were also screened for the presence of phytochemicals with the results showing the presence of flavonoids, alkaloids, tannins and saponins in all the extracts. The study suggested that the selected medicinal plants can be used effectively in the treatment of bacterial infection caused by *Staphylococcus aureus*.

Keywords: *Aloe secundiflora*; *Bulbine frutescens*; *Tagetes minuta*; *Vernonia lasiopus*; *Staphylococcus aureus*; Efficacy test

Introduction

In the past few decades, pharmacological companies have been developing new antimicrobial agents but microbial resistance has been increasing due to the ability of bacterial organisms acquiring resistant genes [1]. Herbal drugs have been used since ancient times to treat diseases and disorders with their antimicrobial properties making them a potent source of new drugs. The use of herbal medicine has been used to positively prevent and control diseases such as, heart disorders, diabetes and other forms of cancer [2]. *Tagetes minuta* belongs to the Asteraceae family which presently comprises of 56 species, 27 biennials and 29 perennials. *Tagetes* species are grown all over the world as multipurpose plants [3]. *Tagetes* species and chemotypes from its genus have been largely examined for biological active metabolites that can be used in industry and medicine [4,5]. The plant extracts from the plants part such as leaves and flowers have been used in treating intestinal and stomach problems [6-8]. *Aloe secundiflora* is common in Kenya; there are about 60 taxa recognized [4]. Other scientific synonyms of *Aloe secundiflora* are *Aloe floramaculata*, *Aloe engleri* and *Aloe marsabitensis* [9]. *Aloe secundiflora* leaf components have been credited for antibacterial, antifungal and antiviral and antihelminthic medicinal properties [9]. *Aloe secundiflora* has been used in treating ailments including; chest problems, polio, malaria and stomach ache by herbalists in the Lake Victoria region [10]. *Bulbine frutescens* belongs in the family xanthorrhoeaceae and sub family asphodeloideae and its members are well known for their medicinal value [11]. It's chiefly found in South Africa with a few species extending to the tropics of Africa and Australia [12]. The leaves of the

plant have been used in the treatment of wound thought to be infected with bacterial pathogens and it has shown antibacterial properties [13]. A decoction of bulbs and roots of some of the species has been used in the treatment of some of the venereal diseases in women and stomach upsets [14]. Vernonieae is a tribe which has about 1300 species and in the family Asteraceae (Compositae) which mostly contains herbaceous plants [15]. *Vernonia lasiopus* decoctions from the stems and leaves have been traditionally been used by herbalists in East Africa to treat, malaria, worms and gastrointestinal problems [16]. Its extracts have also been used in treating some of the sexually transmitted diseases in southern parts of Africa [17]. In the present study, the antimicrobial activity of the medicinal plants extracts against *Staphylococcus aureus* is tested and preliminary screening of the phytochemicals present in each plant.

Materials and Methods

Plant material collection

The fresh plant material of *Aloe secundiflora*, *Bulbine frutescens*, *Vernonia lasiopus* and *Tagetes minuta* was collected at Kenyatta University Arboretum. Voucher specimen was prepared and deposited in the university herbarium in Plant Sciences Department for future reference. The plants were brought to the laboratory and thoroughly washed in running water to remove debris and dust particles and then rinsed using distilled water and finally air dried.

Preparation of plant extract

The air dried plant materials were grinded into powder and soaked in methanol for 72 hours, placed in a Gallenkamp shaker at 65

revolutions per minute. The contents were homogenized and filtered using whatman filter paper no. 1. The filtrate was poured into a round bottom flask and concentrated using a vacuum evaporator and stored in a labelled amber glass bottle at room temperature away from light and heat before being used for antimicrobial efficacy test.

Test bacterial organism

The microorganism used was clinical isolate of *Staphylococcus aureus* obtained from Kenyatta University Health Centre Laboratory, Nairobi. The isolate was tested against methanolic leaf extracts from *Tagetes minuta*, *Aloe secundiflora*, *Bulbine frutescens* and *Vernonia lasiopis*.

Antimicrobial susceptibility testing

The clinical isolate of *Staphylococcus aureus* was concentrated and compared with a 0.5 McFarland standard. Discs of 6 milliliters were prepared from whatman no.1 filter paper. The discs were sterilized by autoclaving. The moist discs were dried on hot air oven at 50°C [18]. The discs were impregnated with the extracts from the highest concentration of 1000 mg/ml to the lowest concentration of 1 mg/ml [19].

The antimicrobial efficacy test was carried out using Kirby Bauer method [20]. Hektoen agar was used in the spread plate technique where *Staphylococcus aureus* was spread using sterilized cotton wool swabs and exposed to extracts impregnated discs in milligrams per microliter from *Aloe secundiflora*, *Tagetes minuta*, *Vernonia lasiopis* and *Bulbine frutescens*.

The discs were placed with equal distance between them on agar plates. Positive control discs containing vancomycin was used against *Staphylococcus aureus*. A negative control of discs impregnated with DMSO and distilled water were also used. The Petri dishes were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimetres and their average determined.

The experiment was carried in duplicates and the diameter of zones of inhibition formed measured. Minimal inhibitory concentration (MIC) was determine using the broth tube [21]. 100 µl of 250 mg/ml of the extracts was added to 100 µl of sterile bacteriological peptone in the first well of the 96 well micro plate and mixed well with a micropipette. 100 µl of this dilution was transferred subsequently to wells two folding each dilution of the original extract. This was done to the extracts of *Aloe secundiflora*, *Bulbine frutescens*, *Vernonia lasiopis*, and *Tagetes minuta*. An inoculum of 100 µl (0.5 McFarland standard) of overnight clinical culture of *Staphylococcus aureus* was added in each of the wells. Triplicate of each micro plate were made and the procedure repeated for each of the test organisms. The plates were then incubated at 37°C for 24 hours. After incubation 40 µl of 0.2 mg/µl of INT were added in each of the wells and the plates examined after an additional 60 minutes of incubation. Growth was indicated by a red colour (conversion of INT to formazan). The lowest concentration at which the colour was apparently invisible as compared to the next dilution was taken as the minimum inhibitory concentration [22]. Minimum bactericidal concentration (MBC) was determined by 100 µl of suspension was taken from micro plate wells that demonstrated no growth and inoculated on agar plates. The plates were incubated at 37°C for 24 hours. In the case where there was no bacterial growth and also not greater than the minimum inhibitory concentration was used to determine the maximum bacterial concentration [22].

Phytochemical screening

Presence of saponins, tannins, flavonoids and alkaloids in the crude extract were determined [23].

Tannins: Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of distilled water. Filtration was carried out after 2 ml of FeCl₃ was added. If there was presence of a blue or black precipitate then it indicated the presence of tannins.

Flavonoids: Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of ethanol and filtered. 2 ml of 1% HCl and magnesium ribbon was added to the filtrate. If there was formation of a pink or red colour it indicated the presence flavonoids.

Alkaloids: Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of methanol and filtered. 1% HCL was added to the filtrate and the solution heated. Mayor's reagent was added drop wise and if there was formation of any colored precipitate it indicated the presence of alkaloids.

Saponins: Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of methanol and filtered. Distilled water was added and shaking done for a few minutes. If there was persistence frothing then it indicated the presence of saponins.

Data Analysis

The data was expressed as means and standard deviations. Statistical analysis for social sciences (SPSS version 21.0) package was utilised in conducting ANOVA test to determine significant differences in antimicrobial activity of selected plant extracts against *Staphylococcus aureus*. The Turkey's post-hoc was utilized to assess the difference within individual means of the zones of inhibition. A P-value ≤ 0.05 was considered statistically significant.

Results

The methanolic leaf extracts from *Tagetes minuta*, *Aloe secundiflora*, *Bulbine frutescens* and *Vernonia lasiopis* all showed a significant antimicrobial activity when tested against the clinical isolate of Gram positive *Staphylococcus aureus*. The extract from *Tagetes minuta* was more active against *Staphylococcus aureus* as compared to others producing the highest average zone of inhibition produced from six replicates (17 ± 1.94 mm). *Bulbine frutescens* extract was the least active producing average zone of inhibition of 12 ± 1.94 mm. The other two extracts from *Aloe secundiflora* and *Vernonia lasiopis* also showed a considerable antimicrobial activity at 13 ± 0.17 mm and 12 ± 1.94 mm respectively. The standard antibiotic used as positive control (Vancomycin and ciprofloxacin) produced zones of inhibition of approximately 25 mm and 22 mm respectively. The negative controls of distilled water and dimethyl sulphoxide did not produce any zones of inhibition. *Tagetes minuta* produced the highest zone of inhibition at least concentration which showed it had a more potent antimicrobial activity as compared to the other extracts (Table 1).

P-value ≤0.001

Key: DMSO4: Dimethyl sulphoxide and Distilled water (negative control), Mean of six replicates ± Standard error, MIC: Minimum inhibitory concentration, MBC: Maximum bactericidal concentration, Vancomycin and Ciprofloxacin (positive control).

All the plants extracts from the plants showed the presence of secondary metabolites being tested for namely saponins, alkaloid, tannins and flavonoids (Table 2).

Plant extracts	MIC (mg/ml)	MBC (mg/ml)	Zone of Inhibition (mm)
<i>Tagetes minuta</i>	8.9	10.0	17 ± 1.94
<i>Aloe secundiflora</i>	10.2	12.9	13 ± 0.17
<i>Bulbine frutescens</i>	10.4	13.9	12 ± 1.94
<i>Vernonia lasiopus</i>	12.2	14.2	14 ± 0.64
Vancomycin	0.0	0.0	25
DMSO4	0.0	0.0	0.0
Distilled water	0.0	0.0	0.0
Ciprofloxacin			22

Table 1: Antimicrobial activity of the plant leaf extracts against *Staphylococcus aureus*.

Plant extracts	Saponins	Tannins	Alkaloids	Flavonoids
<i>Aloe secundiflora</i>	+	+	+	+
<i>Vernonia lasiopus</i>	+	+	+	+
<i>Bulbine frutescens</i>	+	+	+	+
<i>Tagetes minuta</i>	+	+	+	+

Table 2: Phytochemical tests on the plant extracts.

Key: (+) present

Discussion

Staphylococcus aureus are Gram positive bacteria that cause diseases such as skin and soft tissues infections as well as food poisoning and toxic shocks [24]. The rate of mortality associated with *Staphylococcus aureus* in developing world exceeds the one of developed countries [25]. The increasing use of antimicrobials against *Staphylococcus aureus* has led to the development of resistance hence need to develop new antimicrobial agents [26]. Herbal drugs made from medicinal plants have been used from ancient times to treat various diseases and their antimicrobial properties make them a rich source of many potent drugs [2]. The use of herbal medicinal plants has always played a positive role in the control or prevention of diseases such as diabetes, heart disorders and various cancers [27]. Some medicinal plants have been used in production of various drugs as principal raw material for the production of other conventional medicines [28]. In this study, the evaluation of the antimicrobial potency of *Tagetes minuta*, *Aloe secundiflora*, *Bulbine frutescens* and *Vernonia lasiopus* against clinical isolate of *Staphylococcus aureus*. All the plant extracts had some significant antimicrobial activity against *Staphylococcus aureus* with *Tagetes minuta* being more prominent. Similar results were also obtained from antimicrobial activity of methanolic extracts; *Tagetes minuta*, *Aloe secundiflora*, *Bulbine frutescens* and *Vernonia lasiopus*. Medicinal plants have been known to produce an array of phytochemicals with recognized antibacterial activity belonging to chemical structural classes: phenolic, terpenoids,

alkaloids, lectins, polypeptides, and polyacetylenes but the most bioactive constituents are alkaloids, tannins, flavonoids, and phenolic compounds [29,30]. The plant extracts when tested showed the presence of alkaloids, saponins, flavonoids and tannins. The antimicrobial activity of these plant extracts may be due to the presence of secondary metabolites. The findings of the study were similar to others carried out on antimicrobial potency of the plant extracts [31-33]. Other similar studies carried out on Secondary metabolites such as flavonoids, saponins, tannins and alkaloids have shown to have antimicrobial activity against both Gram positive and Gram negative bacteria [16,29,34-37].

Conclusion

In conclusion, all the plant extracts leaf extracts showed a considerable antimicrobial activity against *Staphylococcus aureus* which is a Gram positive bacteria. This shows that the extracts from the plants could be used as an antimicrobial agent against *Staphylococcus aureus* and other bacterial pathogens of the same nature (Gram positive). There is also need to further purify the primary phytochemicals found into specific bioactive components to determine the compounds responsible for this antimicrobial activity. This will aid in the provision of a natural source of treating diseases caused by this bacterial pathogen and others of its kind that have been gradually developing resistance against conventionally used antibiotics.

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