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Phytochemical Analysis and Antibacterial Activity of the Kenyan Wild Orchids

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ABSTRACT

The current study evaluated the antibacterial activity of dichloromethane and methanol (DCM-MeOH) extracts of four Kenyan orchid species against three selected bacterial strains. Extracts of E1 (*Ansellia Africana*), E2 (*Trydactylescottelli*), E3 (*Polystachyabella*) and E4 (*Liparis bowkeri*) were screened for antibacterial activity against *staphylococcus aureus*, Bacillus subtilis and *Pseudomonas aeruginosa* using agar disc diffusion. Ampicillin was included as a positive control. Qualitative analysis revealed the presence of flavonoids, saponins, alkaloids, tannins, terpenoids, steroids and glycosides. *Trydactyle scottelli and Polystachyabella* extracts revealed a more substantial presence of tannins and steroids, respectively, compared to others. All extracts showed varying levels of antibacterial activity against the test bacteria. However, *Polystachyabella* and *Liparis bowkeri* against *Bacillus subtilis* and *Ansellia Africana* against *Pseudomonas aeruginosa* exhibited higher activities similar to that of Ampicillin. The study further showed that the DCM-MeOH extracts of the four orchids contain potential compounds that should be screened for conventional management of bacterial infections.

1. Introduction

Excessive usage of antibiotics in humans and veterinary medicine has led to the emergence of antibiotic resistance that requires novel antibiotics. As a result, many diseases such as pneumonia, tuberculosis, gonorrhoea, and salmonellosis are becoming harder to treat with the currently available antibiotics [1, 2]. This has been attributed to antimicrobial resistance amongst common bacterial pathogens that threaten this therapeutic aid [2]. Antibiotic resistance is an immediate threat to the treatment of infectious diseases as the development of new antimicrobial agents is declining [2, 3].

Currently, there is an increasing interest in plants as a source of drugs because of their pharmacological potency and extended traditional usage[4-6]. In addition, plant phytochemicals have displayed potential antibacterial properties against sensitive and resistant pathogens [4, 7]. It is, therefore, imperative to screen plant extracts for novel antimicrobial properties as a possible source for new drug development [4, 8]. One family of plants of interest in searching for plant-based therapeutics and antimicrobial compounds is Orchidaceae[9].

The Orchidaceae is one of the largest and most diverse families of flowering plants comprising more than 28,000 accepted species spanning 763 genera [10]. These species are absent only from polar and desert regions but are particularly abundant worldwide in wet tropics [11].

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The plants have beautiful flowers in shape, size, and colour, making them an essential economic resource in global horticulture. In addition, they are crucial medicinally and also serve as ecological indicators [12, 13]. Orchids are grown for ornamental purpose, used as herbal medicines food, and has cultural value by different tribes globally [9].

Medicinal orchids contain phytochemicals such as alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes and terpenoids, which are present in leaves, roots, pseudobulbs, and flowers [14]. However, there are limited reports on the bioactive compounds and their pharmacological properties of the Kenyan wild orchids. In this regard, it was critical to evaluate the phytochemical and antibacterial properties of four different species of orchids.

2. MATERIALS AND METHODS

2.1 Sample collection and authentication

Different orchid plants were sampled from the Mau forest complex (MFC). MFC is amongst the richest yet least studied in orchid diversity [15], containing some endemic and threatened species in the IUCN Red List. A taxonomist identified the plants before being transported to the National Museums of Kenya (NMK) for further processing.

2.2 Sample preparation

The collected samples were washed to remove mud and dust then air-dried for 68 h in a well-ventilated room and ground using an electric laboratory mill (Christy & Norris Ltd., Chelmsford, England). A total of 10grams of powdered sample was extracted in 100ml dichloromethane (DCM) and methanol at a ratio of 1:1. The sample was then soaked in DCM-methanol for 48 h and filtered using a clean gauze followed by Whatman No. 1 filter paper (Whatman, England). Re-maceration of the residue was done for 48h and filtered. The filtrates–were pooled together in a round-bottomed flask and evaporated to dryness by a rotary evaporator (model No.). The crude extracts were further dried in a vacuum drier to expel any solvent remnants and stored at -20^0 C for future use. Extraction yield (%) was calculated as previously described [16].

$$\text{Percentage Yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \times 100$$

2.3 Qualitative Phytochemical Analysis

The dichloromethane (DCM): methanol extracts were assessed for the presence of the various phytochemicals of interest using the standard methods[17-19].

2.3.1 Test for alkaloids

Dilute HCL acid onto 1.5 g of extract. Drops of Wagner's reagent (Iodine in Potassium Iodide) were then added. Brown/reddish precipitate showed the presence of alkaloids.

2.3.2 Test for Terpenoids

A 2.0 ml of chloroform was mixed with 5 ml aqueous plant extract, evaporated on a water bath and then boiled with 3 ml of concentrated H_2SO_4. A grey colour formed which showed the entity of terpenoids.

2.3.3 Test for saponins

Using the form test where 2 ml of water was added to 1.5 g of extract and mixed well by shaking. Consistent foam development for 7 minutes indicated the presence of saponins.

2.3.4 Test for steroids

Based on Salkowski's Test, 1.5 g of extract, 2 ml of chloroform and 2 ml of concentrated sulphuric acid were added and mixed thoroughly. Greenish-yellow fluorescence at the red layer of chloroform and acid indicated the presence of steroids.

2.3.5 Test for glycosides

This was done by–adding 1ml distilled water to 1.5 g of extract and shaking to mix thoroughly. Further, the aqueous solution of sodium hydroxide was added dropwise. The yellow colour indicated the presence of glycoside.

2.3.6 Test for tannins

According to the gelatin test, 1.5 g of extract was added to 2 ml of 1% gelatin solution comprising sodium chloride. The formation of white precipitate exhibited the presence of tannins.

2.3.7 Test for flavonoids

This was done using an Alkaline reagent test where4 drops of Sodium hydroxide solution were carefully added to1.5 g of extract. Formation of intense yellow colour that disappeared upon dropwise addition of dilute HCL acid—denoted the presence of flavonoids.

2.4 Antibacterial assay

Antibacterial assays of the DCM: methanolic extracts of different plants was performed using disc diffusion methods Mueller Hinton Agar (MHA) plates[20]. The test bacteria *(Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa)* from the stock cultures were grown overnight at 37°C in MHA and used as the inoculum. The bacterial cultures were adjusted to 0.5 McFarland's standard to achieve a 1.5 ×108 CFU/ml concentration in physiological saline. They then used to evenly lawn Mueller–Hinton agar plates using sterile cotton swabs [21]. Inoculated plates were air-dried in the laminar airflow hood for 15 min and then used for the sensitivity test. The discs, impregnated with a series of plant extract dilutions, were placed on the Mueller–Hinton agar surface, with each test plate comprising eight discs equidistant from each other. Ampicillin 10µg/ml (Oxoid) standard was used as positive control while the negative control consisted of 10% DMSO. All the tests were performed in triplicates, and plates were incubated at 37°C for 24 h. After incubation, plates were observed to form a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The diameter zone of inhibition (DZI) was observed and measured in mm.

2.5 Statistical Analysis

The diameter zones of inhibition (DZI) readings were recorded in M.S. Excel and analyzed using Prism software version 8.0. The results were expressed as mean ± standard error of the mean (SEM). One-way ANOVA and Tukey's posthoc test separated and compared means. P-values less than 0.05 were considered statistically significant.

3. RESULTS

Qualitative analysis for plant secondary metabolites in selected wild orchids revealed the presence of flavonoids, saponins, alkaloids, tannins, terpenoids, steroids and glycosides in varying concentrations.

Flavonoids and glycosides were lowly presents in all the plants except *Ansellia Africana* and *Trydactylescottelli.* The low presence of saponin was recorded in all the plants compared to tannin that occurred strongly and moderately in *Trydactyle scottelli* and *Ansellia Africana*, respectively.

All the tested secondary metabolites were present in *Polystachyabella compared to* Terpenoids was only absent in *Liparis bowkeri.* The least number of plant metabolites were recorded in *Trydactyle scottelli* with flavonoids, alkaloids, steroids and glycosides missing.

Dichloromethane: Methanol extraction produced different extraction yields that ranged from 2.5% (*Trydactylescottelli*) to 3.25 % (*Polystachyabella*).

The five DCM: methanol extracts showed mean values of diameter zones of inhibition (DZI) against *Staphylococcus aureus* ranging from 7.67 mm to 9.33 mm (Fig. 1). The highest mean DZIwas recorded in E3 (9.33 mm) followed by E2 (9.0 mm), E3 (8.33 mm) and E4 (7.67 mm) compared to the control (13.0 mm). There were no differences between the mean DZIof the extracts (E1, E2, E3 and E4) against *S. aureus*. However, the 4 plant extracts' DZI was significantly different from the positive control (p <0.05).

Table 1. Qualitative Phytochemicalprofiles of selected Kenyan wild orchids

S/N	Orchid species	Flavonoids	Saponins	Alkaloids	Tannins	Terpenoids	Steroids	Glycosides	Extract yield (%)
1	*Ansellia africana*	-	+	+	++	+	++	-	3.0
2	*Trydactylescottelli*	-	+	-	+++	++	-	-	2.5
4	*Polystachyabella*	+	+	++	+	+	+++	+	3.25
4	*Liparis bowkeri*	+	+	++	+	-	++	+	2.75

Strongly present: +++, moderately present: ++, lowly present: +, absent: -.Extract yield iswas expressed as percentage (%).

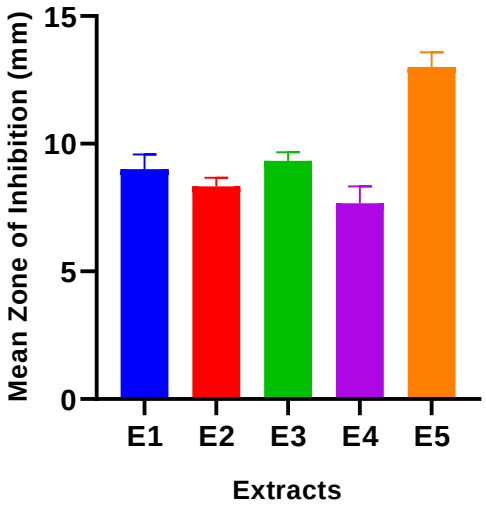

Fig. 1. Mean zone of inhibition of extracts on Staphylococcus aureus. E1: Ansellia africana, E2: Trydactylescottelli, E3: Polystachya bella, E4: Liparis bowkeri, E5: Control (Ampicillin)

As indicated in Figure 1, DCM: methanol extracts of the plants tested against *Bacillus subtilis* produced mean DZI that varied from 9.67 mm (E1 and E2) to 11.67 mm (E4). E4 (11.67 mm)had the highest DZI, followed by E3 (11 mm) compared to E1 and E2, which recorded similar values of 9.67 mm each. Mean values of DZI for E1 and E2 were statistically different from each other (p <0.05). However, the DZI for E3 and E4 were not significantly different from the positive control (Ampicillin)(Figure 2).

Mean DZI against E1 (12.67 mm), E2 (10.33 mm), E3 (11.33 mm) and E4 (9.0 mm) showed significant variation from each other (p <0.05) against *Pseudomonas aeruginosa.* E1 (12.67 mm) had the highest DZI with no significant differences than the positive control (14.33 mm) (Figure 3).

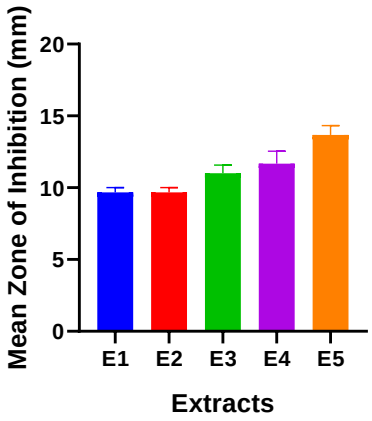

Fig. 2. Mean zone of inhibition of extracts on *Bacillus subtilis.* E1: *Ansellia africana,* E2*: Trydactylescottelli,* E3: *Polystachya bella*, E4: *Liparis bowkeri,* E5: Control (Ampicillin)

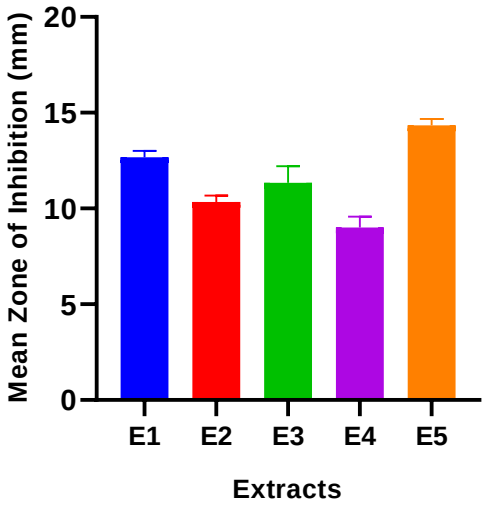

Fig. 3. Mean zone of inhibition of extracts on *Pseudomonas aeruginosa*. E1: *Ansellia africana,* E2*: Trydactylescottelli,* E3: *Polystachya bella*, E4: *Liparis bowkeri,* E5: Control (Ampicillin)

4. DISCUSSION

Extracts E1, E2, E3 and E4 demonstrated varying activity levels against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The inhibition observed on the tested strains of bacteria shows that orchid extracts have a broad spectrum of activity. Broad-spectrum activity of plant extracts against different strains of bacteria has been reported in other studies [21, 22]. The DZI values for all the extracts against *Staphylococcus aureus* and extract E1 and E2 against *Bacillus subtilis* were > 10 mm. The zone of inhibition values > 10 mm for medicinal orchids is considered active [23]. The basis of variation in sensitivity has been attributed to the intrinsic nature and combinations of phytochemicals present in each extract [24].

The extracts had flavonoids, saponins, alkaloids, tannins, terpenoids, steroids and glycosides with varying degrees of product yield (%). Total antibacterial activity is a function of the extraction yield of plant material and DZI[25]. Differences in extraction yield are due to polarities of the compounds present in the plants and have been reported elsewhere in medicinal plants [26].

The performances of E3 (*Polystachya Bella*) and E4 (*Liparis bowkeri*) extracts against *Bacillus subtilis* were similar to the positive control (Ampicillin). Additionally, E1 (*Ansellia Africana)* had DZI comparable to the control against *Pseudomonas aeruginosa*.

E3 and E4 contained flavonoids and glycosides absent in the other plant extracts. Similar studies have reported that glycosides and flavonoids are the most common constituents in orchids[27, 28]. Flavonoids exhibit remarkable antibacterial activities against Gram +ve and Gram –ve bacteria such as *Pseudomonas aeruginos*us and *Staphylococcus aureus*[8, 29, 30]. In addition, flavonoids cause bacterial cell-membrane damage and inhibit various synthases involving nucleic acid and cell envelope synthesis[31]. Recent studies by Yuan et al. (2021) and Kiani and Jabeen, 2019 indicated that the lipophilicity of plant flavonoids is a crucial factor for their inhibitory activities against Gram-positive bacteria. Apart from orchids, other plant extracts with appreciable quantities of flavonoids include *Bacteria nigritiana, Moringa oleifera, Cordia millenii, Afrormosia laxiflora* and *Sacoglottis gabonensis*[32].

Some orchids are also crucial for their glycosidal value, where different glycosides have been reported to be present[33, 34]. Glycoside inhibits bacterial growth, particularly Gram-positive such as *Staphylococcus aureus*[35]. In addition, it affects the growth of bacteria by inhibiting RNA nucleic acid synthesis [36].

5. CONCLUSIONS

Among the orchid species tested, *Trydactylescottelli and Polystachya Bella* extract revealed a more substantial presence of tannins and steroids, respectively, compared to the others. Extracts of *Polystachya bella&Liparis bowkeri* against *Bacillus subtilis* and *Ansellia Africana* against *Pseudomonas aeruginosa* exhibited activities compared to Ampicillin. The active compounds in the orchids highlighted in this study require further characterization and

testing for their effective therapeutic exploitation in conventional medicine. Further, all the tested extracts revealed the presence of varying levels of antibacterial properties against bacterial infections.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

There are no conflicts of interest among the authors regarding publication of this article.

Ethical Approval

This article does not contain any studies with human participants or animals

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