

Phytochemical Analysis and Antibacterial Activity of selected Kenyan Wild Orchids

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Abstract

The current study evaluated 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 stronger presence of tannins and steroids respectively compared to the others. All extracts showed varying levels of antibacterial activity against the test bacteria. However, extracts of *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 bioactive compounds that should be further analysed for conventional and traditional management of bacterial infections.

Introduction

Excessive usage of antibiotics in humans and veterinary medicine has led to the emergence of antibiotic resistance that requires the use of novel antibiotics. A large number of diseases such as pneumonia, tuberculosis, gonorrhoea, and salmonellosis are becoming harder to treat with the currently available antibiotics (Prestinaci et al., 2015, Munita and Arias, 2016). This has been attributed to antimicrobial resistance amongst common bacterial pathogens that are now threatening this therapeutic aid (Munita and Arias, 2016). Antibiotic resistance is an immediate threat to the treatment of infectious diseases as the development of new antimicrobial agents is declining (Conly and Johnston, 2005, Munita and Arias, 2016).

Currently, there is an increasing interest towards plants as source of drugs because of their pharmacological potency and long traditional usage (Sonter et al., 2021). Plant phytochemicals have displayed potential antibacterial properties against sensitive and resistant pathogens (Sonter et al., 2021). It is therefore imperative to screen plant extracts for novel antimicrobial properties as a possible source for a new drug development (Khameneh et al., 2019, Sonter et al., 2021). One family of plants of interest in the search for plant-based therapeutics and antimicrobial compounds is Orchidaceae (Panda and Mandal, 2013).

The Orchidaceae is one of the largest and most diverse families of flowering plants comprising of more than 28,000 accepted species spanning 763 genera (Zhang et al., 2018). These species are absent only from polar and desert regions but are particularly abundant in the wet tropics worldwide (Ramya et al., 2020). The plants have beautiful flowers in terms of shape, size and colour making them an important economic resource in the global horticultural. They are important medicinally and also serve as ecological indicators (Newman et al., 2009, Fonge et al., 2019). Orchids are grown for ornamental purpose, used as herbal medicines, food, and has cultural value by different tribes globally (Challe and Struik, 2008, Panda and Mandal, 2013). Medicinal orchids contain phytochemicals such as alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes and terpenoids, which are present in leaves, roots, pseudobulbs, and flowers (Wati et al., 2021). There are limited reports on the bioactive compounds and its 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.

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 terms of orchid diversity (Kawaka et al., 2014), containing some endemic and threatened species in the IUCN Red List. The plants were field identified by a taxonomist 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 10 grams 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 vacuum drier to expel any solvent remnants and stored at -20⁰ C for future use. Extraction yield (%) was calculated as previously described (Bhatia and Narain, 2010).

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

2.3 Qualitative Phytochemical Analysis

The dichloromethane (DCM): methanol extracts were assessed for presence of the various phytochemicals of interest using the standard methods (Pochapski et al., 2011, Gul et al., 2017, Gaya et al., 2013).

2.3.1 Test for alkaloids: Dilute HCL acid was added 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 H₂SO₄. A grey color formed which showed the entity of terpenoids.

2.3.3 Test for saponins: This was done using the foam test where 2 ml of water was added to 1.5 g of extract and mixed well by shaking. Consistence 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 completely. 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 shaken to mix completely. Further, aqueous solution of sodium hydroxide was added dropwise. A yellow colour indicated the presence of glycoside.

2.3.6 Test for tannins: According to gelatin test, 1.5 g of extract was added to 2 ml of 1% gelatin solution comprising Sodium chloride. Formation of white precipitate exhibited the presence of tannins.

2.3.7 Test for flavonoids: This was done using Alkaline reagent test where 4 drops of Sodium hydroxide solution were carefully added to 1.5 g of extract. Formation of intense yellow colour that disappeared upon drop wise addition of dilute HCL acid denoted presence of flavonoids.

2.4 Antibacterial assay

Antibacterial assays of the DCM: methanolic extracts of different plants was performed using disc diffusion method in Mueller Hinton Agar (MHA) plates (Humphries et al., 2018). 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 concentration of 1.5 × 10⁸ CFU/ml in physiological saline and then used to lawn Mueller–Hinton agar plates evenly using sterile cotton swabs (Sieber et al., 2020). 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. A standard of Ampicillin 10µg/ml (Oxoid) was used as a positive control while the negative control comprised 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 for the formation of 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 data on diameter zones of inhibition (DZI) readings were recorded in MS 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 post-hoc test was used to

separate and compare means. *P* values less than 0.05 were considered statistically significant.

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 present in all the plants except *Ansellia africana* and *Trydactyle scottelli*. 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 tested plant secondary 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% (*Trydactyle scottelli*) to 3.25 % (*Polystachyabella*).

Table 1: Qualitative Phytochemical profiles of selected Kenyan wild orchids

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

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

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 DZI was 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 significant differences between the mean DZI of the extracts (E1, E2, E3 and E4) against *S. aureus*. However, the DZI of the 4 plant extracts were significantly different from the positive control ($p < 0.05$).

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 that 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 compared to the positive control (Ampicillin).

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 compared with the positive control (14.33 mm).

Discussion

Extracts E1, E2, E3 and E4 demonstrated varying levels activity 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 (Sieber et al., 2020, Abate et al., 2021). 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 (Bhatnagar et al., 2017). The basis of variation in sensitivity has been attributed to intrinsic nature and combinations of phytochemicals present in each of the extract (Aqil and Ahmad, 2003).

The extracts had flavonoids, saponins, alkaloids, tannins, terpenoids, steroids and glycosides with varying degree of product yield (%). Total antibacterial activity, is a function of the extraction yield of plant material and DZI (Elisha et al., 2017). Differences in extraction yield are due to polarities of the compounds present in the plants, and have been reported elsewhere in medicinal plants (Sheneni Victor et al., 2018, Nguyen and Eun, 2011).

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 that were absent in the other plant extracts. Similar studies have reported that glycosides and flavonoids are the most common constituents in orchids (Minh et al., 2016, Stavenga et al., 2021). Flavonoids exhibit remarkable antibacterial activities against Gram +ve and Gram –ve bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Wu et al., 2013, Khameneh et al., 2019, Kiani and Jabeen, 2019). The flavonoids cause bacterial cell-membrane damage, inhibit various synthase involving the nucleic acid and cell envelope synthesis (Yuan et al., 2021). Recent studies by Yuan et al. (2021) and Kiani and Jabeen, 2019 indicated that the lipophilicity of plant flavonoids is a key factor for their inhibitory activities against Gram-positive bacteria. Apart from orchids, other plant extracts with appreciable quantities of flavonoids include *Barteria nigritiana*, *Moringa oleifera*, *Cordia millenii*, *Afrormosia laxiflora* and *Sacoglottis gabonensis* (Ezeonu and Ejikeme, 2016).

Some orchids are also important for their glycosidal value where different kinds of glycosides have been reported to be present (Marjoka et al., 2016, Nugraha et al., 2020). Glycoside inhibits bacterial growth particularly those of Gram positive such as *Staphylococcus aureus* (Kriker et al., 2014). It affects growth of bacteria by inhibiting the RNA nucleic acid synthesis (Kim et al., 2002, Oyama et al., 2016).

Among the orchid species tested, *Trydactylescottelli* and *Polystachya bella* extracts revealed stronger presence of tannins and steroids respectively compared to the other. 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 and traditional medicine. Further, all the tested extracts revealed the presence of varying levels of antibacterial properties against bacterial infections.

Declarations

Ethics approval and consent to participate

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

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PC, HG and NK carried field and laboratory experiments, AA and KO conducted data analysis, HM and FK designed and conceived the study.

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Declarations

Not applicable.

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Figures

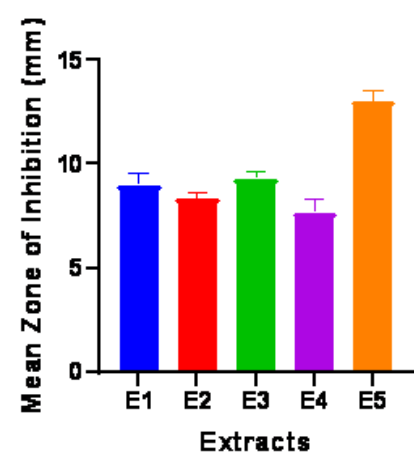


Figure 1

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

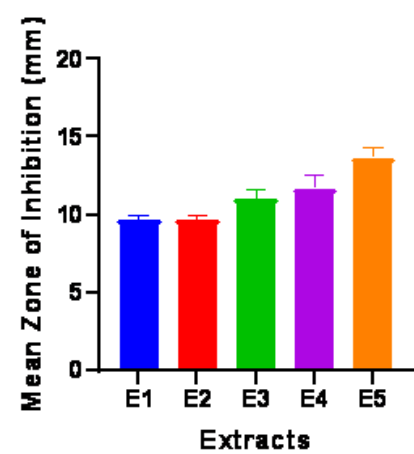


Figure 2

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

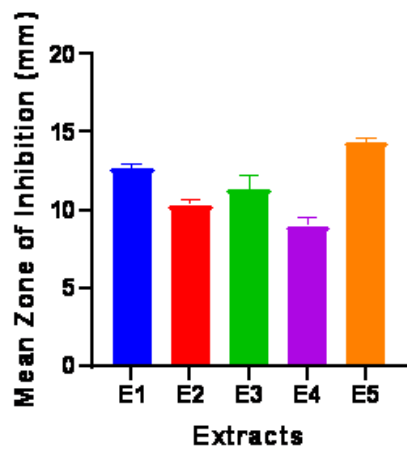


Figure 3

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