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Comparative Phytochemical and Antimicrobial Properties of Two Cultivars of *Catharanthus roseus* L. {G.} Don on *Escherichia coli* and *Candida albicans*

David Mutisya Musyimi* and Marble Namarobe Namnabah

Department of Botany, School of Physical and Biological Sciences, Maseno University, Private Bag, Maseno, Kenya

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Abstract

Medicinal plants have served as sources of medicine to treat and suppress the diseases, because many pathogens are gaining resistance to the current synthetic drugs. In addition, high cost and adverse side effects are commonly associated with popular Synthetic drugs. Therefore, there is need for continuous search for new drugs in order to overcome this emerging resistance. Plants synthesize bioactive compounds which are of great potential in agriculture, antimicrobial and anti-insect activity. The concentration of bioactive compounds in each plant species depends on the environmental conditions, age of the plant, relative humidity of harvested materials and method of extraction. Little is known on the phytochemical and antimicrobial potential of Alba and Rosea cultivars of *Catharanthus roseus* ethanol extracts. The leaves of Alba and Rosea cultivars were investigated for their phytochemical and antimicrobial properties. The study was conducted at Maseno University, Kenya. Plant Leaves were collected around Maseno University. Leaves of Alba and Rosea cultivars of *Catharanthus roseus* were air-dried in the shade, thereafter crushed into powder and ethanol extraction done using the Rotary evaporator. Antimicrobial activity of the pathogenic microorganisms was *Candida albicans* and *Escherichia coli*. The paper disc diffusion method was used for antimicrobial tests. Different concentrations of ethanol leaf extracts which consisted of 2.5, 5and 7.5 mg/mL with three replications. Sterile water was used a control. The data on growth inhibition were subjected to analysis of variance (ANOVA) using SAS statistical package. Treatment means were separated and compared at p = 0.05. Phytochemical analysis revealed the presence of tannins, flavonoids, terpenoids, saponins, alkaloids and phenols in the leaf extract except steroids and glycosides. The ethanol leaf extracts were active against *Candida albicans* and *Escherichia coli*. Alba leaves extracts showed higher inhibitory zones compared to Rosea leaves. The observed differences in antimicrobial activity could be due to differences in cell wall synthesis, structure and composition. The results of present study further confirm the use of these plants traditionally for the treatment of different ailments.

1. Introduction

The use of plants for medicine has been practiced for many years (**Kokwaro, 2009; Musyimi *et al.*, 2008**). Man has used various parts of plants in the treatment and prevention of various ailments (**Mohammed *et al.*, 2011**). Plants produce secondary metabolites such as alkaloids, cyanogenic glycosides, glucosinolates, flavanoids, saponins, steroids and terpenoids (**Shalini and Sampathkumar, 2012**). Tannins possess anti-fibrotic effects (**Chuang *et al.*, 2011**). These bioactive compounds are of great potential in agriculture, antimicrobial and anti-insect activity (**Emitaro *et al.*, 2020b**). Endophytes synthesize bioactive compounds or their precursors which help them protect the host plants against pathogens (**Emitaro *et al.*, 2020a**). Previous studies have shown that endophytic microbial communities within medicinal plants have a great potential as producers of novel bioactive compounds and hence high potential for agricultural and pharmaceutical (**Köberl *et al.*, 2013; Rai *et al.*, 2014**). Tanshinones have diverse pharmacological activities such as anticancer, antidiabetes, cardioprotective effects and neuro-protective activity (**Teimoori-Boghsani *et al.*, 2020**). Microbial infections pose a health problem throughout the World, and plants are a possible source of antimicrobial agents (**Burapadaja and Bunchoo, 1995; Adenisa *et al.*, 2000**). Medicinal plants contain active principles which can be used as an alternative to cheap and effective herbal drugs against common bacterial infections. The curative properties of medicinal plants are attributed to the presence of various phytochemicals (**Sheeraz *et al.*, 2013**). The concentration of bioactive compounds in each plant species depends on the environmental conditions, age of the plant, relative humidity of harvested materials and method of extraction (***Emitaro et al.*, 2020a**). Phytochemicals distribution patterns in plants may differ within and between geographical locations due to differences in environmental conditions. Phytochemicals have the ability to protect humans against various diseases (**Rumzhum *et al.*, 2012**). Phytochemicals such as anthraquinones, tannins, terpenoids, and glycosides have antimicrobial activities and antioxidant properties (**Zheng *et al.*, 2001; Tiwari *et al.*, 2011; Benhammou *et al.*, 2013**). The

*Corresponding author: dmusyimi@maseno.ac.ke
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medicinal value of a plant depends on the chemical constituents in it that produce definite physiological action on the human body (**Aiyelaegbe and Osamudiamen, 2007; Musyimi *et al.,* 2008**). Screening of herbs for pharmacological activities and phytochemical constituents is one of the active fields of research round the world today (**Khair-ul-Bariyah *et al.,* 2012; Sheeraz *et al.*, 2013**). *Catharanthus roseus* (L.) is an important medicinal plant of the family Apocynaceae is used to treat many of the fatal diseases (**Jaleel *et al.,* 2009**). There are about two common cultivars of *C. roseus* which are named on the basis of their flower color that is the pink flowered 'Rosea' and the white flowered 'Alba' (**Sain and Sharma, 2013**). *C. roseus* is extensively cultivated in northern India for its ever increasing demand in pharmaceutical and medical industry **(Patil and Ghosh, 2010; Nayak *et al*., 2006)**. *Catharanthus roseus* has been used in folk medicine to treat sore throat, mouth ulcer, diabetes, high blood pressure, muscle pain and cancer treatment (**Devi *et al*., 2013; Sain and Sharma, 2013**). Most pathogens are developing resistance against many of the currently available antimicrobial drugs (**Patil and Ghosh, 2010; Devi *et al.,* 2013; Ramya *et al*., 2008**). Resistance in pathogens has increased at high rate and multi drug resistant microorganisms have exacerbated the situation (**Nino *et al*., 2006; Schinor *et al*., 2007**). Emerging and re-emerging infections and microbial drug-resistance pose a challenge to the global public health. Plants provide unique elements which are indispensable for novel drug discovery (**Essawi and Srour, 2000; Goyal *et al.,* 2008; Khalil, 2012**). There is an urgent need to search and develop cheaper plant based drugs. Recent attention has been on compounds. The increased preference of herbal medicine has consequently propelled the search for pharmaceutical remedies against different ailments from plants (**Tugume *et al.,* 2016**). *C. albicans* cause infections that range from superficial infections of the skin to life-threatening systemic infections (**Mayer *et al*., 2013**). Multi-drug resistant diarrhoeagenic *E. coli* have been isolated from children (**Vila *et al.,* 1999**). According to **Mathekaga and Mayer (1998)** screening methods provide preliminary observations necessary to select crude plant extracts for further chemical and pharmacological investigations. Little research has been done on phytochemical and antimicrobial properties of *Catharanthus roseus* in Kenya. This study aimed at investigating the phytochemical and antimicrobial properties of leaf extracts of two cultivars of *Catharanthus roseus* on *Escherichia coli* and *Candida albicans*.

2. Material and methods

2.1 Field collection

Catharanthus roseus leaves of the two cultivars were collected around Maseno University Siriba campus, near Maseno Anglican Hospital. They were identified at the Maseno University Herbarium. The dirt adhered to the specimen was cleaned off by shaking the plant as well as washing using tap water then they were sterilized using methylated spirit. They were spread on the sterilized laboratory bench to air dry for three weeks at room temperature, away from direct sunlight.

2.2 Extraction

After collection and identification, the leaves were air-dried in the laboratory for two weeks and the dried leaf material were weighed using electronic weighing balance, and grinded with electric grinder into fine powder (**Balaabirami and Patharajan, 2012**). Fifty (50g) dry powder of each variety were macerated cold in200ml of 70% ethanol for one week in a maceration tank at room temperature. Filtration was done using the whatmann no. 1 filter papers and the filtrate further transferred to the rotary evaporator (manufactured by Tokyo Rikakinah Co. Ltd-Eyela of type SB-1000 and operates at AC 230V, 50Hz, 1.1KVA) where ethanol evaporated at 78^0C leaving semisolid substances which were left to solidify and dry. After drying, the masses of the dry extracts were determined and the extracts kept safe at room temperature until usage.

2.3 Culturing of microorganisms

The microorganisms used in the antimicrobial activity of *Catharanthus roseus* were *Escherichia coli* and *Candida albicans.* The microbes were obtained from Maseno University Botany Laboratory. They were cultured using the Nutrient Agar (N.A) for bacterium and Potato Dextrose Agar (PDA) for fungi according to **Chon and Nelson (2004).**Test cultures were prepared by transferring a loop full of *Escherichia coli* from stock culture nutrient broth and incubated at 37^0C for 24h. *Candida albicans* were transferred into freshly prepared dextrose agar plates and incubated at 25^0C.

2.4 Comparative antimicrobial screening

The dry extracts were dissolved in sterile distilled water, to prepare solutions of 2.50, 5.0, and 7.5 mg/mL of different concentration of each extract. Circular discs of 6mm diameter were cut from Whatman no. 1 filter paper in the laboratory using a paper punch. They were dipped in the known concentrations of the plant extracts and allowed to absorb the plant extracts according to **Musyimi *et al.* (2008)**. Approximately 1×10^5cells/ml suspension of the *C. albicans* and *Escherichia coli* were aseptically inoculated on PDA and on nutrient agar petri dishes respectively. Sterile paper discs were then soaked in the prepared extracts of *Catharanthus roseus* leaf extracts and were transferred to the inoculated agar media, 3 discs each petri dish. They were sufficiently spaced to prevent the resulting zones of clearing from overlapping. The petri dishes were incubated for 48hrs at 27ºC. A paper disc impregnated in sterile water was used as control experiment (**Balaabirami and Patharajan, 2012**). The zone of inhibition was determined using a transparent ruler and a caliper to the nearest millimeter from the lower surface of the Petri dishes.

2.5 Phytochemical analysis

Phytochemical screening was done according to **Trease and Evans (1983)** and **Harbourne (1973)**.

Test for alkaloids
Two grams of the extract were extracted by warming it for 2 minutes with 20ml of 1% H_2SO_4 acid in a 50ml conical flask on a water bath, with intermittent shaking. One drop of Meyer's reagent was added to 0.1ml supernatant in a semi-micro tube. A cream precipitate indicated the presence of alkaloids.

Test for flavonoids
5 milliliters of dilute ammonia solution were added to a portion of the aqueous filtrate of the extract followed by addition of concentrated H_2SO_4. A yellow colouration indicated the presence of flavonoids.

Test for tannin
About 0.5 g of the dried powdered samples was boiled in 20ml of water in a test tube and filtered through Whatman No. 42 filter paper. A few drops of 0.1% ferric chloride were added. A brownish green or a blue-black coloration indicated the presence of tannins.

Test for phenols
Ferric chloride test was carried out where the extract was diluted to 5ml with distilled water. Then, a few drops of neutral 5% Ferric chloride solution were added. A dark green or a blue-black colour indicated the presence of phenolic compounds.

Test for steroids
Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2ml H_2S0_4. Colour change from violet to blue or green indicated the presence of steroids.

Test for saponins
About 2 g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. Ten millilitres of the filtrate were mixed with 5ml of distilled water and shaken vigorously to form a stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously, and then was observed for the formation of emulsion.

Test for terpenoids
Five millilitres of each extract was mixed with 2ml of chloroform, and concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration that formed at the interface indicated the presence of terpenoids.

Test for Cardiac glycosides
Five ml of extract was treated with 2ml of glacial acetic acid containing a drop of $FeCl_3$ solution. This was then underplayed with 1ml conc. H_2SO_4. A brown ring of the interface indicated a deoxy-sugar characteristic of cardiac glycosides.

2.6 Data analysis

The data collected were subjected to analysis of variance (ANOVA) using SAS statistical package. The treatment means were separated and compared at ($p<0.05$).

3. Results

Phytochemical screening of leaf extracts of the two cultivars of *Catharanthus roseus* confirmed the presence of alkaloids, terpenoids, saponins, tannins, phenols and flavonoids in the ethanol leaf extracts, but lacked steroids and glycosides (Table 1).

Table 1 Phytochemical screening of secondary metabolites in the leaf extracts of two cultivars of *Catharanthus roseus*

Phytochemicals	Alba leaves	Rosea leaves
alkaloids	+	+
steroids	-	-
terpenoids	+	+
saponins	+	+
tannins	+	+
phenols	+	+
flavonoids	+	+
glycosides	-	-

+: Present, -: Absent

The two plant cultivars extracts were found to possess antimicrobial activities on *E. coli* and *C. albicans* (Table 2 and Table 3). The Alba cultivar leaf extracts showed higher inhibitory activity on the bacterial strain (*E. coli)* compared to the Rosea cultivar leaf extract. The leaf extract was found to be most active against *C. albicans* (inhibition zone of 9mm) compared to *E. coli* (inhibition zones of 8.99 mm).

Table 2 The growth inhibitory effect of plant leaf extract of *Catharanthus roseus* cultivars *on Escherichia coli* and *Candida albicans*

Catharanthus roseus Cultivars	*Microbe*	*Extract concentration (mg/mL)*	*Zone of inhibition (mm)*
Alba	*E. coli*	0.0	6.00±0.00
		2.5	8.00±0.51
		5.0	12.10±2.00
		7.5	16.89±1.48
Alba	*C. albicans*	0.0	6.00±0.00
		2.5	9.00±0.84
		5.0	9.33±0.84
		7.5	11.33±1.17
Rosea	*E. coli*	0.0	6.00±0.00
		2.5	6.11±0.40
		5.0	8.11±0.48
		7.5	8.78±0.78
Rosea	*C. albicans*	0.0	6.00±0.00
		2.5	9.00±0.77
		5.0	9.67±2.22
		7.5	11.67±1.35

Table 3 Comparative Antimicrobial effects of different concentrations of leaf extract of *Catharanthus roseus cultivars* on *Escherichia coli* and *Candida albicans*

Concentration of Extracts (mg/mL)	*Diameter of inhibition (mm)*
0	6.00d
2.5	8.19c
5.0	9.64b
7.5	12.17a
LSD	1.3802
Microbes	
E. coli	8.99a
C. albicans	9.00a
LSD	0.976
Cultivars	
Alba	9.8321a
Rosea	8.1667b
LSD	0.976

Means with the same letter down the column are not significantly different. Data presented are means of three replicates.

4. Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. Plants offer the local population with immediate and accessible therapeutic products (**Mwaura *et al.,* 2020; Bruck *et al.*, 2004**). Plants and their secondary metabolites have shown great potential as antibacterial and antifungal sources (**Bikash *et al.,* 2011**). In Kenya, traditional medicines play a major role in primary healthcare and upkeep of rural communities (**Kokwaro, 1988; Kisangau and Kokwaro, 2004**). Over 70% of the Kenyan population relies on traditional medicine as their primary source of healthcare (**Odera, 1997**). Phytochemical screenings help to reveal the chemical nature of plant constituents (**Enwuru *et al., 2008***). The results indicated that the two cultivars of *Catharanthus roseus* extracts contained alkaloids, terpenoids, saponins, tannins, flavonoids and some phenolic compounds. Steroids and cardiac glycosides were absent in the leaves. These results are in agreement with those of **Phani *et al.* (2013); Sheeraz *et al.* (2013)** and **Giri *et al.* (2012)**. Composition of secondary metabolites varies from species to species, climatic

conditions and the physiological state of developments of the endemic plants (**Musyimi *et al*., 2008; Hussain and Deeni, 1991**). The biochemical composition of plants is the most common parameter used for the characterization of plants (**Deepak *et al.,* 2009**).The presence of these bioactive compounds in ethanolic leaf extracts may account for the antimicrobial activity. The detected compounds in this study have been vastly reported for their antimicrobial activities (**Tiwari *et al*., 2011; Sheeraz *et al*., 2013)**. The curative properties of medicinal plants have been attributed to the presence of alkaloids, flavonoids, phenols, saponins, steroids in plants (**Britto and Sebastian, 2011**). Phenolic compounds are most abundant plant metabolites with a variety of antimicrobial properties (**Audu *et al*., 2007**). Alkaloids do not show direct antimicrobial actions but strengthens the immune system. If the immune system is too weak or the organism is too virulent then certain other medication, along with these compounds are given to show significant antimicrobial activity (**Patil and Ghosh, 2010**). Flavonoids inhibit many bacterial strains and important enzymes. Tannins have astringent properties which accelerate the healing of wounds and inflamed mucous membranes. Tannin compounds inhibit the growth of microorganisms (**Chung *et al.,* 1998**). Plants with tannins are used to treat non-specific diarrhea and inflammation of the mouth (**Shohel *et al.,* 2014**). The biological function of alkaloids, saponins and their derivatives are very important (**Stary, 1998**). Saponins dissolve in water to form foamy solutions and because of surface activity, some drugs containing saponins have a very high long history of usage. Preliminary screening tests may be useful in detecting the bioactive principles (**Doss *et al.,* 2009**). The antimicrobial activities of ethanol extract may be due to the presence of tannins, triterpenoids and flavonoids (**Mamtha *et al.,* 2004**). Differences in antimicrobial activity of medicinal plants are obviously related to differences in their contents of active compounds. These compounds having minimum side effect and can be easily substituted for antibiotics. Therefore, the presence of these phytochemicals could to some extent justify the observed antimicrobial activities in the current study. This may be due to structural differences between bacterial and fungal agents. Phytochemical compounds are responsible for antimicrobial activity against stomach pain and diarrhea pathogens (**Sahleand and Okbatinsae, 2017**). This study demonstrates that ethanol leaf extract of *Catharanthus roseus* are effective against *Candida albicans*. *Candida albicans* are very resistant fungi (**Khalil, 2012**). The results have shown that there was a significant difference between the plant cultivars. Alba cultivar was highly effective. However, these differences that were observed among the microbial activities could be due to the difference in the chemical composition of the plant extracts as revealed by phytochemical analysis. The differences could also be attributed to the differences in susceptibility of the test microorganisms to the phytochemicals and differences in cell wall structure and composition. In fact, it has been reported in other studies (**Tekwu *et al.,* 2012; Zavala *et al.,* 1999**). Plant extracts often show a higher activity against bacteria compared to fungi, and this may partly be due to differences in the cell wall synthesis and structure. The susceptibility of *E. coli* and *C. Albicans* to the ethanol extract is a clear indication that these plants can be further exploited as a potential source of antibacterial and antifungal compounds.

5. Conclusion

In this study ethanol leaf extracts of Alba and Rosea varieties of *Catharanthus roseus* inhibited the growth of *E. coli* and *C. albicans*. The findings indicate that ethanol leaf extract of *Catharanthus roseus* possess potential antibacterial and antifungal activity. This study has also revealed the presence of phytochemical constituents in the leaves except the steroids and cardiac glycosides in the *Catharanthus roseus.* The appreciable antimicrobial activities of ethanol extract noted in this study may be due to the presence of tannins, triterpenoids and flavonoids. The findings from this study support the traditional use of this plant. Moreover, the findings of this study add value to the traditional uses of these plants. In conclusion, the two plant cultivars leaves may be reliable sources of antimicrobials which can be used the development of novel drugs and the treatment of multi drug resistance pathogens*.* More research is needed to draw the comparison on effectiveness of various plant part extracts.

Declaration of interest

The authors declare that they have no conflicts of interest.

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