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Phytochemical Screening and *in vitro* Antimicrobial Activity of *Dracaena Afromontana* Leaves

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ABSTRACT

Dracaena afromontana is a native species grown in the high mountain rainforest of Rwanda. This plant finds many traditionally medicinal uses in the treatment of chest pain, dermatitis, liver diseases and malaria. In this report, the dried Bay leaves were extracted with n-hexane, ethyl acetate and methanol by maceration giving 0.717 g (1.434%), 1.457 g (2.914%) and 6.319 g (12.64%) respectively. The extracts were concentrated for further phytochemical screening and evaluated for antimicrobial activity against *E. coli* and *S. aureus* using well diffusion method. In addition, the *dracaena afromontana* extracts were analyzed using thin layer chromatographic separation techniques leading to the identification and characterization of bioactive compounds including, terpenoids, flavonoids, tannins, phenols, saponins, reducing sugar and quinones. The results from TLC indicate that the higher retention factor ($Rf_2 = 0.77$) was obtained *via* the use of ethyl acetate, whereas n-hexane gave a retention factor ($Rf_3 = 0.558$). On the other hand, the methanol extract did not show any separation. The antimicrobial assay for the extracts was carried out using Lurial broth agar and Manitor salt over lay method. The findings of antimicrobial assay showed that methanolic extract of *dracaena afromontana* leaves has an antibacterial activity against the gram negative bacteria *E.coli* with the zone of inhibition of 16 mm, while the antibacterial activity of ethyl acetate extract against *E. coli* showed a smaller inhibition zone of 10 mm for diluted inoculums (10^{-2} and 10^{-1}). For the gram positive bacteria *S. aureus* the inhibition zone by *Dracaena afromontana* was insignificant, whereby the methanolic and ethyl acetate extracts showed a maximum inhibition zone of 4 mm and 3 mm respectively. The n-hexane extract did not show any antibacterial activity against both pathogenic organisms.

Key words: *dracaena afromontana*, phytochemical screening, anti-microbial activity, *E. coli*, *S. aureus*.

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INTRODUCTION

In the recent years, human beings have used different plants for the treatment of various disorders (Azwanida, 2015; Sofowora, 1996). A number of tropical countries, especially those of Africa, use different plant parts for the treatment of different diseases as herbal drugs (Banso & Adeyemo, 2006). Many plants contain a variety of secondary metabolites including tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils (Cowan, 1999). Numerous studies for new drugs discovery from plant products involve the investigation of traditional medicine, routine biological screening, toxicological evaluation and the development of bioassays (Williamson, 2001). Various reports showed that multidrug resistant microbes are becoming considerable substantial global health threats (Telci, Toncer, & Sahbaz, 2006). The discovery of new pharmaceuticals requires sound knowledge for new classes of safe and more effective antimicrobial agents by acting with different mechanisms. A number of plants containing secondary compounds could possess some of these ideal preservative characteristics mainly due to their antioxidant, anti-microorganisms and other biological potentials (Bakkali *et al.*, 2008). Secondary metabolites show other various applications such as flavours, perfumes, insecticides and herbicides (Croteau, Kutchan, & Lewis, 2000; Dewick, 2002). Most bio-molecular drugs derived from alkaloids, glycosides, steroids, terpenoids are among the category of various pharmaceuticals (Kar, 2003).

Today, plants largely contribute in the treatment of different illness through the use of both herbs and different plant natural products. The determination of plants whose product can inhibit the bacterial growth is of high importance for the protection of human health and ecology in general (Erdogrul, 2002). Recent reports revealed that plant materials are the main basis of all medical system worldwide, especially in many countries from Africa and Asia, due to the fact that many people from the latter continents rely on traditional healers as their primary health care needs. This practice led to various serious problems as it was done without any knowledge regarding the scientific information about the used plant as well as the doses to be used by patient based on the gender,

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age and state of life (Chase, Reveal, & Fay, 2009; Mbugua & Moore, 1996). Most traditional healers used the bark and roots for the liver disease and malaria alleviation; thus its removal from the tree is very destructive to the plant and the society at large (Kayonga & Habiyaremye, 1987). The plant *Dracaena Afromontana* is dominated by its major characteristics classifying it as a shrub tree with narrowly lanceolate leaves, inflorescence pendulous (Kokwaro, 1976). This plant is subjected to high risk of loss as forests habitat in East African Countries are under severe threat from conversion to farming, and infrastructure development, and subpopulations of this species might have been lost (Bogawski *et al*., 2019; Lulekal *et al*., 2013).

In Rwanda, the two native species of the above-mentioned plant including *dracaena afromontana* and *dracaena steudneri* are sometimes grown through seeds propagation and cutting stem to make field boundaries and graves, as well as for ornamental purposes (Bloesch, Troupin, & Derungs, 2009). In four East African Countries; Tanzania, Uganda, Burundi and Rwanda, its roots and bark are variably used as medicine for chest pains and rheumatism treatment (Wilkin, 2008). Herein, we report the extraction of extracts from leave *of dracaena afromontana* plant, whereby the obtained extracts were analyzed for the presence of bioactive compounds according to standards methods (Ngbede, Yakubu, & Nyam, 2008). Anthropogenic activities *via* industrial pollution and sometimes non standard products; air and polluted water, microbe and parasite infections can lead to many diseases (Bloesch *et al*., 2009). The literature shows that more studies should be done on this plant as very little research has been conducted on it. Therefore, this study reports the phytochemical screening of *dracaena afromontana* mild leaves, qualitative analysis of their main constituents and the *in vitro* antibacterial activity of its leaves extracts.

MATERIAL AND METHODS

Preparation of Plant Material

The leave of the plant (Figure 1) were collected, washed with distilled water to remove all impurities and then air dried to remove the water content. The dried plant material was ground to a fine powder using an appropriate machine. The powder was kept in a cool and dry place for further uses.

Preparation of Crude Extracts

The preparation of crude extracts was performed using maceration to extract natural products using three solvents including n-hexane, methanol and ethyl-acetate. This technique consists of soaking plant material in the appropriate solvent for a sufficient time by daily agitation, and the content was then filtered and purified. For maceration, the coarsely powdered plant drug was kept in contact with the solvent in a closed container for 2 days with frequent agitation until soluble matter was dissolved. The mixtures were prepared by soaking 50 g of *dracaena afromontana* leave powder in 250 ml of ethyl acetate for 48 hours, 50 g of *dracaena afromontana* leave powder soaked in 350 ml of methanol for 48 hours and 50 g of *dracaena afromontana* leave powder soaked 48 hours in 200 ml of n-hexane.

Phytochemical Screening Assay

The extracts were screened for the presence of major phytochemicals using standard qualitative methods (Evans, 1989; Harborne, 1998). Powder of the *dracaena afromontana* was applied in different screening methods to identify compounds present in the leave of the latter plant. The qualitative analysis of phytochemicals methods was used to detect the presence of alkaloid (Doughari, 2006; Doughari & Manzara, 2008; Fransen, Crosbie, & Edmonds, 2001), tannins (Fransen *et al*., 2001), quinone, flavonoids, anthocyanin, terpenoid (Mithraja *et al*., 2012), saponins (Das, 2012), reducing sugar and phenolics (Ishtiaq *et al*., 2014).

Thin Layer Chromatography

Thin layer chromatography (TLC) and column chromatography are common and useful separation techniques for a mixture of biomolecules or organic molecules (Moody & Harwood, 1989). The cruds extract was subjected to TLC to identify how many components available in these extracts using methanol, diethyl ether, n-hexane, and mixture of solvents in different proportions (Fenimore & Davis, 1981). Then, different components were visualized using UV Lamp, and each component was characterized by its retention factor.

$$Rf = \frac{D1}{D2}$$

Where; R_f: Retention factor, D1: distance traveled by the spot, D2: distance traveled by solvents.

Culture Media Preparation and Antibacterial Activity Essay

The lurial agar medium preparation was performed whereby the mass of 17.2 g of lurial broth powder was mixed with 15 g of agar powder in 1000 ml of water, and then put in autoclave at 121°C for 15 min. On the other hand, the Manitol salt agar medium was prepared by mixing 111.02 g in 1000 ml of

distilled water and boiling to dissolve the medium completely. The controls were prepared without extract. Ethanol (99.8%) was used as a negative control while standard commercial antibiotic (ampicillin at the conc. 0.1g/ml) was used as a positive control. The analysis of antimicrobial activity from *dracaena afromontana* extracts was performed using disk diffusion method (Bauer *et al*., 1966), and the extracts were used in conjunction with control of ampicillin to compare the degree of inhibition and effect on bacterial growth. The plates were then incubated at 37°C for 24 hours, and after incubation period, the plates were examined for inhibition zone and effect on bacteria. From the stock culture of *E. coli* and *S. aureus*, the Lurial Agar medium (LA) and Manitol Salt Agar (MSA) were used. Then, for sterilization the mixture was put in autoclave at 121°C for 15 min. Three types of inoculums; undiluted and diluted inoculums (10^{-1} and 10^{-2}) were used for antibacterial test against *E. coli* and *S. aureus* bacteria, and the test organisms were maintained on Manitol Salt Agar medium and Lurial Agar medium cultures. Each plate was impregnated by 100 µl of the extract solution at concentration 0.1 mg/ml. The inhibition zones were measured with a ruler and recorded, and the appeared clearance zones were an indication of a positive antibacterial activity of the extracts.

Table 1 **Yield of Crude extract of *dracaena afromontana* leaves**

No	Solvent (ml)	Mass of Extract (g)	Yield in Percentage (%)
1.	Methanol	6.319	12.638
2.	N-Hexane	0.717	1.434
3.	Ethyl-acetate	1.457	2.914

RESULTS AND DISCUSSIONS

The results of the extraction are summarized in Table 1. The obtained data showed that n-hexane extract has a smaller yield (1.434%) comparing to that of ethyl acetate (2.914%) and methanol (12.368%). The powder of *dracaena afromontana* leaves were used to analyze the presence of different phytochemicals (Table 2), and the data shows that the extract of *dracaena afromontana* leaves in n-hexane contains terpenoids, flavonoids, saponins, tannins, reducing sugars, quinones, phenols, and are represented by a positive signs (+).

The analysis using thin layer chromatography (TLC) helped to determine the number of components present in the crude extract. Since the silica gel is polar, the more polar compounds were retained on the plate with a low retention factor, whereas the less polar compounds moved fast with the highest retention factor, resulting in the separation of different constituents of the crude extracts. The choice of a solvent which gave well separated compounds was done, and after visualizing the fractions using UV-lamp. The result showed that n-hexane extract had 3 compounds; ethyl acetate extract had 2 compounds with different retention factors while the methanol extract did not give clear separation of the compounds (Table 3).

Lurial agar disc diffusion tests were performed to study the antibacterial activity of *dracaena afromontana* leaves extracts (Figure 2), and three extracts including *Dracaena afromontana* methanolic (A) extract, *Dracaena afromontana* ethyl acetate extract (C), and *Dracaena afromontana* n-hexane extract (D) were used. The diameter of the inhibition zones that have been developed due

Table 2 **Results of phytochemical screening of *dracaena afromontana***

No	Tested content	Reagent used	Expected results	Obtained Results	Observation
1.	Alkaloids	Draggendorf	White orange to Red precipitate	No precipitate	-
		Mayer	White-yellow precipitate	No precipitate	
		Wagner	Red-brown precipitate	No precipitate	
2.	Saponin	Saponin test	Formation of emulsion	Emulsion	+
3.	Flavonoids	Willstrater	Orange or red (Cherryred)	Orange color	++
4.	Tannins	Saline gelatin	Brownish green or blue black	Brownish green	+
5.	Quinone	Diluted NH_3	Red color	Red color	+
6.	Anthocyanins	Acidic medium (HCl)	Red color	Black green	
		Basic medium (NH_3)	Blue	Black green	-
7.	Terpene	Salkowisky test	Reddish brown	Reddishbrown	++
8.	Glacial glucosides	Glacial acetic acid	Blown ring Greenish ring (at interface)	No interface	-
9	Reducing sugar	Fehling's solution	Brick red precipitate	Red precipitate	+
10	Phenols	Ferric Chloride	Bluish green	Black green	++

Key: (++) Strong positive test, (+) Weak positive test, (-) Negative test (absent)

Table 3 Results of thin layer chromatography of different extracts

No	Plant extract	Retention Factor
1.	n-hexane extract	$Rf_1 = 0.168$
		$Rf_2 = 0.480$
		$Rf_3 = 0.558$
		Rf_2 (yellow green) = 0.77
2.	Ethyl acetate extract	Rf_1 (orange) = 0.36
3.	Methanolic extract	No separation

Table 4 Inhibition zones developed by the control (ampicillin) and the extracts against *E. coli*

Extract	Culture media	Inoculation bacteria (*E. coli*)		Inhibition zone (cm) by extract (1 drop)	Inhibition zone (cm) by ampicillin (1 drop)
		Concentration (diluted in peptone)	Quantity		
Dracaena A. methanolic extract (A)	L.A	Undiluted	0.1ml	0.8	1.2
	L.A	10^{-1}	0.1ml	0.9	1.4
	L.A	10^{-2}	0.1ml	1.6	2.1
Ethyl acetate extract (C)	L.A	Undiluted	0.1ml	0.2	0.6
	L.A	10^{-1}	0.1ml	1	1.2
Dracaena A. n-hexane extract (D)	L.A	Undiluted	0.1ml	-	0.3

Table 5 Inhibition zones developed by the control (ampicillin) and the extracts against *S. aureus*

Extract	Culture media	Inoculation bacteria (*S. aureus*)		Inhibition zone (cm) by extract (1 drop)	Inhibition zone (cm) by ampicillin (1 drop)
		Concentration (diluted in peptone)	Quantity		
Dracaena A. methanolic extract (A)	MSA	Undiluted	100 µl	0.2	1.4
	MSA	10^{-1}	100 µl	0.3	1.5
	MSA	10^{-2}	100 µl	0.4	1.6
Dracaena A. Ethyl acetate extract (C)	MSA	Undiluted	100 µl	0.1	0.6
	MSA	10^{-1}	100 µl	0.3	1.2
Dracaena A. n-hexane extract (D)	MSA	Undiluted	100 µl	-	0.2

to control and extracts are shown in Table 4 and 5. The findings indicated that the methanolic extract of *Dracaena Afromontana* leaves at the concentration of the extract of 0.1 mg/ml had an antibacterial activity against *Escherichia coli,* diluted in peptone water (10^{-2}), with zone of inhibition 1.6 cm (Figure 1a) and 0.9 cm at the concentration of 10^{-1} of the inoculum (Figure 1b). The activity of the ethyl acetate extract against *E. coli* led to inhibition

Figure 1 *Dracaena afromontana* leaves.

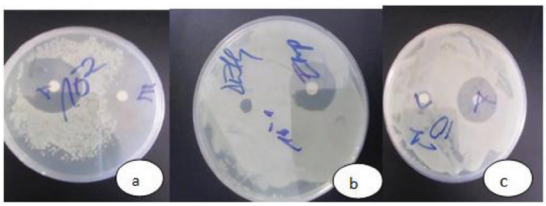

Figure 2 Antimicrobial activity of Methanol extract (a and b) and Ethyl acetate extract

zone of 1 cm at diluted bacteria in peptone water (10^{-1}) (Figure 1c). For the gram positive bacteria *S. aureus*, the inhibition zone by *Dracaena afromontana* was insignificant, whereby the inhibition zone produced by methanolic extract was 0.4 cm at the concentration of 10^{-2} of the inoculum, and that of ethyl acetate extracts showed a maximum inhibition zone of 0.3 cm at the concentration of 10^{-1} of the inoculum. The n-hexane extract did not show any antibacterial activity against both tested pathogenic organisms. The inhibition zone of the positive control; ampicillin has a diameter higher than that of the plant extracts, due to the fact that the antibiotic used is commercial purified.

CONCLUSION

The bioactive compounds occurring in plant material in various plants are multi component mixtures, and their separation and characterization continue to be a challenging issue. Their separation, screening and purification require sound skills in order to isolate bioactive compounds. In this study, the analysis showed the presence of seven phytochemical bioactive compounds from the extracts of n-hexane, methanol and ethyl acetate of the *dracaena afromontana* leaves mild. Furthermore, the biological study concerning the antimicrobial assay of the extract showed the moderate high activity against

the pathogenic bacteria. The biological activity of this plant may be associated with the presence of the seven identified bioactive compounds and/or in combination with the rest that we could not be able isolate. We are still working on other parts of the plant to study their phytochemical screening, test on their toxicity, biological activity and pharmacological properties.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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