Metal Analysis, Phytochemical and Antibacterial Investigation of *Crinum Macowanii* Bulb

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***Abstract*—** *Crinum macowanii* Baker is a medicinal plant native to Southern Africa. It is commonly known as the Cape coast lily. Phytochemical screening of the crude extracts showed the presence of tannins, reducing sugars, flavonoids, steroids, alkaloids, saponins and cardiac gylcosides. The plant was extracted using methanol/chloroform 50:50 (v/v) and pressurized hot water extraction technique (PHWE). Antibacterial activities of the crude extracts were analyzed using disc diffusion and Minimum Inhibitory Concentration (MIC). The methanol/chloroform extracts showed noteworthy antibacterial activity against *Mycobacterium smegmatis* (MIC 0.125mg/mL), *Bacillus cereus* (MIC 0.5mg/mL), S*taphylococcus epidermidis* (MIC 0.0625mg/mL) and the PHWE extract showed antibacterial activity against *B. cereus* (MIC 8mg/mL), *S. epidermidis* (MIC 16mg/mL), *E. cloacae* (MIC 16mg/mL) and *P. aeruginosa* (8mg/mL). Metal analysis using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) detected elements such as Calcium, Potassium, Sodium, Chromium, Nickel and Iron. This provides some validation of the traditional medicinal uses of the plant against the treatment of itchy rashes, boils, acne, backache and venereal disease, inflamed sores, swelling of the body, urinary tract problems and is also used to increase lactation in women and cows.

***Index Terms*—** Anti-bacterial, *Crinum macowanii*, Pressurized Hot Water Extraction (PHWE), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

I. Introduction

Medicinal plants have been used traditionally for centuries as folk medicines [1]. The therapeutic effects of the plants are based on their chemical makeup [2]. The World Health organization (WHO) reported that up to 80 % of the world's population depend on some form of traditional medicine for their primary healthcare needs [3]. This is likely due to their easy access, affordability and cultural significance [4]. Medicinal plants have been reported to be important and rich sources of lead therapeutic compounds in the development of novel drugs, this could be due to elements present in the plants that promote good health [2], [5]. *Crinum macowanii* Baker from the *Amaryllidaceae* family is indigenous to Southern Africa and it grows widely over the subcontinent [6]. It is called *`umduze'* by the Zulu tribe in South Africa and has always been used traditionally for different applications. The bulb is used to treat itchy rashes, boils, acne, backache, venereal disease, inflamed sores, swelling of the body, urinary tract problems and is also used to increase lactation in women and cows. The leaves are used as bandages. Both the leaves and bulb are used to make protective charms [7, 8]. Due to the widespread use of *Crinum* species in traditional medicines for the treatment of aliments associated with bacterial infections, there are additional bacterial pathogens that this plant has not been investigated on. As such, this necessitates the need to further study the antibacterial properties of the plant on neglected bacterial pathogens. There is also a need to study the metal content and concentrations since medicinal plants are very important and need to be screened for toxic trace elements to compile a scientific data base for traditional practitioners and pharmaceutical industries. In this paper, organic extracts and pressurized hot water extracts of *C. macowanii* bulbs were investigated for their antibacterial activity against fourteen selected pathogens. A phytochemical screening was also done to identify the various classes of secondary metabolites that could be present. A metal analysis was done to check for metal toxicity and possible health benefits the metals have to support the scientific validation and effectiveness of the medicinal uses of the plants.

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II. Materials and Methods

A. Plant material collection and extraction

The bulbs of *Crinum macowanii* were purchased at Faraday Muthi market in Johannesburg South Africa in January 2015. A voucher specimen (no. BTNST01) is available at the UJ herbarium. The plant was subsequently extracted using solvents according to the method described by [9] and Pressurized Hot Water Extraction (PHWE) according to [10].

B. Phytochemical screening of Crinum macowanii

Phytochemical screening is used to check for possible phytochemical groups present in the crude extract. A method described by [9] and [11] was followed.

C. Antibacterial screening of crude bulb extracts

The following bacterial strains were obtained from Davies Diagnostics: *Bacillus cereus* (ATCC10876), *Bacillus subtilis* (ATCC19659), *Enterococcus faecalis* (ATCC13047), *Staphylococcus epidermidis* (ATCC14990) and

Staphylococcus aureus (ATCC25923). *Enterobacter aerogenes* (ATTC13048), *Enterobacter cloacae* (ATCC13047), *Escherischia coli* (ATCC25922), *Klebsiella oxytoca* (ATCC8724), *Klebsiella pneumonia* (ATCC13882), *Proteus mirabilis* (ATCC7002), *Proteus vulgaris* (ATCC6380) and *Pseudomonas aeruginosa* (ATCC27853). *Mycobacterium smegmatis* (MC 155) was obtained from the University of the Witwatersrand, Johannesburg (Centre of Excellence in Biomedical TB Research). Methods by [12] and [13] were followed for disc diffusion and Minimum Inhibitory Concentrations (MIC) respectively.

D. Trace metal analysis of Crinum macowanii bulbs by inductively coupled plasma optical emission spectrometry (ICP-OES)

The determination of metals was done according to a method described by [14].

III. RESULTS

The bulbs showed a strong result in the test for alkaloids, confirming that the plant is rich in this specific group of compounds. In the test for saponins a lot of persistent froth also formed, but the test is not quantitative, so no conclusions could be made on the amount of saponins present. Tannins, flavonoids, steroids and cardiac glycosides could also be detected in the bulbs.

TABLE I
PHYTOCHEMICAL SCREENING OF CRUDE BULB EXTRACT OF *CRINUM MACOWANII*

Chemical Compound	Observation	Results
Tannins	Faint green colour	++
Flavonoids	Yellow coloration	+++
Steroids	Red ring	+++
Reducing Sugars	Dark green colour	++
Alkaloids	Turbidity/ Precipitation	+++++
Saponins	Persistent froth	++
Cardiac glycosides	Brown ring at interface	++

Indicates presence of phytochemicals
++= shows low prevalence
+++ = shows moderate prevalence
+++++ = shows high prevalence
- = indicates absence

In the disc diffusion experiments *C macowanii* showed the highest antibacterial activity against *Bacillus subtilis* with a 12.67 mm zone of inhibition for the solvent extract and 6.12mm inhibition for *Staphylococcus aureus* for the PHWE extract. The lowest inhibition in the disc diffusion was 8.3mm for *Bacillus cereus* in the solvent extract and 4.45 for *Pseudomonas aeruginosa* in the PHWE extract.

The plant extract displayed no activity against *Enterococcus faecalis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherischia coli*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Mycobacterium smegmatis* for the disc diffusion. From the results it is observed that the Gram-negative bacteria strains *Enterobacter cloaca*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella oxytoca* and *Enterobacter aerogenes* had not been inhibited by the crude extract during the disc diffusion method but inhibition could be observed by the microdilution method, this could be because the polarity of the natural compounds can affect the diffusion of compounds onto the culture, whereby medium having compounds with less polarity diffusing slower than more polar compounds [15]. From the microdilution results, the highest inhibition of the solvent extract was 0.125mg/Ml against *Mycobacterium smegmatis* and 8mg/mL for the PHWE extract for both *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*

TABLE II
ANTIBACTERIAL EVALUATION OF *CRINUM MACOWANII* BULBS.

Bacteria species	Disc diffusion Zone of inhibition (mm)			MIC (mg/mL)	
	Control	Solvent extract	PHWE extract	Solvent extract	PHWE extract
B. cereus	26.67	**8.30**	2.45	**0.5**	16.00
B. subtilis	0.00	**12.67**	**5.67**	16.00	>16
E. faecalis	22.33	0.00	0.00	8.00	>16
M. smegmatis	23.33	0.00	0.00	**0.125**	>16
S. aureus	24.67	**10.67**	**6.12**	8.00	>16
S. epidermidis	22.33	2.67	0.00	**0.0625**	**8.00**
E. aerogenes	27.00	0.00	0.00	16.00	>16
E. cloacae	11.60	0.00	0.00	16.00	16.00
E. coli	24.00	0.00	0.00	8.00	>16
K. oxytoca	26.00	0.00	0.00	4.00	>16
K. pneumoniae	26.67	0.00	0.00	>16	>16
P. mirabilis	24.00	0.00	0.00	>16	>16
P. vulgaris	26.67	0.00	0.00	>16	>16
P. aeruginosa	30.67	**8.67**	**4.45**	16.00	**8.00**

0.00=No Inhibiion

Metals such as Potassium 45.844mg/kg, Calcium 19.714mg/kg, and Sodium 18.902mg/kg were detected at moderately high amounts in the bulbs and these are essential nutrients to the body. Gallium, Rhodium and Indium could not be detected from the bulbs. Rhodium and Indium are toxic.

TABLE III
ELEMENTAL COMPOSITION IN CRINUM MACOWANII BULBS (MG/KG DRY WEIGHT DW) DETECTED BY INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES).
RESULTS ARE PRESENTED AS MEAN ± SD

Analyte	Metal element and the analytical wavelength (nm)	ICP-OES
Calcium	Ca 317.933	**19.714**± 1.465
Cadmium	Cd 226.502	0.126113±0.005156
Chromium	Cr 283.563	**5.607**± 0.114101
Copper	Cu 324.754	0.048316± 0.00462
Iron	Fe 239.562	**5.662**± 0.01699
Gallium	Ga 141.444	ND
Mercury	Hg 253.652	0.544336±0.016136
Potassium	K 766.491	**45.844**± 4.349
Manganese	Mn 257.611	0.059166±0.001086
Sodium	Na 588.995	**18.902**± 0.943072
Nickel	Ni 221.648	**5.626**± 0.004035
Lead	Pb 172.680	0.873992±0.019682
Rhodium	Rh 343.489	ND
Strontium	Sr 421.552	**3.83**± 0.012028
Zinc	Zn 206.200	0.037933±0.007622
Indium	In 230.606	ND

ND = Not detected

IV. DISCUSSION

The phytochemical screening in the present study revealed the presence of steroids, flavonoids, tannins, saponins, alkaloids, reducing sugars and cardiac glycosides. The different phytochemicals have been reported to have therapeutic properties, hence the use of *Crinum macowanii* bulbs for medicinal purposes. From the phytochemical test done, steroids were observed in moderate concentration. Steroids such as androgens, estrogens and progestogens are terpenoid lipids and function as hormones for controlling metabolism, for developing and functioning of the sexual organs and for biological differences between the sexes [16]. Progesterone regulates female reproductive functions such as induction of ovulation, facilitation of implantation, maintenance of early pregnancy and lobular-alveolar development in preparation for milk secretion [17]. This supports the traditional use of the bulb to increase lactation in women and cows as reported by [7]. Flavonoids were observed in moderate concentration, from the test done. According to [36], flavonoids have been reported to have bactericidal effects on several strains of bacteria by complexing with the cell wall and binding to adhesins. Flavonoids compounds have been reported to have anti-bacterial activity on bacterial strains include *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aerginosa* [18] and [19], which are causative agents of most nosocomial infections [20] hence this justifies the use of the bulb for the treatment of itchy rashes, boils and acne,

Low concentration of tannins was observed from the phytochemical tests done. According to [21], it was reported that traditionally medicinal plants containing tannins are used as astringents, against diarrhea since astringents tighten and contracts human tissue. Tannins are also known to form a protective layer over exposed tissue that prevents further infection of the wound resulting in the wound healing internally [22]. The presence of tannins in the plant justifies the traditional use of the leaves from the plant as bandages on wounds and cuts. Saponins were observed in low concentration from the test done. Saponins stimulate mechanisms responsible for wound healing by changing extracellular matrix metabolism [23]. *Staphylococcus aureus* and *Enterococcus faecalis* were inhibited by Diosgenyl 2-amino-2-deoxy-beta-D-glucopyranoside in a model used for wound healing [24]. This justifies the traditional uses of the leaves from the plant as bandages. High concentration of alkaloids was observed from the bulbs plant and this supports literature as stated by [25], [8], [26]. Quinoline, an alkaloid and its derivatives has been reported to show antibacterial activity against *Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus* [27] and skin conditions such as wound infections, skin infections and boils are commonly caused by these bacteria [28], [29]. This will explain the use of the plant traditional to treat itchy rashes, boils and acne. [30] Reported that about 40 alkaloids, especially isoquinolines have been found to have anti-inflammatory properties. This justifies the traditional use of the plant for the treatment of inflamed sores. Low concentrations of cardiac glycosides were observed in the test done. Cardiac glycosides have been reported to have antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumonia* which are the causative agents of nosocomial infections and wounds infections [31]. This supports the traditional use of the plant for the treatment of itchy rashes, boils, acne and inflamed sores [6]. Environmental factors such as water and light availability can affect the concentration of phytochemicals present in plants [32]. [33] Suggested that phytochemicals are degraded during postharvest storage and the growing of medicinal plants in controlled environment systems will help in improving phytochemical production, which will improve the production of pharmacological drugs.

C macowanii showed inhibitory action against both Gram-positive and Gram–negative bacteria, with more Gram-positive bacterial species susceptible to the antibacterial compounds in the bulbs. Most Gram-negative bacteria are known to have multidrug-resistant pumps which force out drugs out of the outer membrane hence inhibition of most species of Gram positive bacteria as stated by [34]. From the results obtained, *Pseudomonas aeruginosa* was the only Gram-negative specie that showed susceptibility to the bulb extract with an inhibition of 8.67mm and 4.45mm for solvent extract and PHWE extract respectively for the disc diffusion method. *Pseudomonas aeruginosa* (ATCC27853) is known not to be inhibited by *Crinum* species since it's able to transform lycorine into its inactive metabolite 2-O-demethylungiminorine as reported by [34], which in this case is the opposite. *P. aeruginosa* is known to cause nosocomial infections involving the respiratory tract, the urinary tract and wounds [35]. With the results obtained, this will support the traditional use of the bulb for the treatment of urinary tract problems. The MIC values for most of the Gram-negative bacteria could not be determined since the values were above 32mg/mL which was the highest concentration tested for both extracts. Bacterial species with

the lowest MIC concentrations with the solvent extract were *Mycobacterium smegmatis* 0.125mg/mL, *Bacillus cereus* 0.5mg/mL and *Staphylococcus epidermidis* 0.0625mg/mL. The lowest inhibitory concentrations for the PHWE extract were 8 mg/mL for both *Staphylococcus epidermidis* and *Pseudomonas aerginosa*. PHWE method is known to be selective in the extraction of compounds, especially polar compounds [36]. Even though water has physical and chemical properties like no other solvent, when it is at a high temperature it tends to mimic the dielectric constants to that of the solvents like ethanol and methanol [36]. It would be expected that the PHWE extract inhibit bacterial species same way as the solvent extract which from Table II was not the case. Factors such as the age of the plant, the amounts of bioactive compounds extracted affect the yield and effectiveness of bioactive compounds [37]. *Mycobacterium smegmatis* is a Gram-positive bacterium which shares some virulence gene homology as *Mycobacterium tuberculosis* which a causative agent for most case of tuberculosis [38]. With an inhibition value of 0.125mg/mL, this can help us combat the virulent *Mycobacteria* since 100 µg/ml of crude extract is required for as an anti-infective drug as stated by [39]. *Bacillus cereus* a Gram positive bacterium, which is a causative agent for most foodborne diseases and is considered a contaminant if isolated form clinical specimen such as blood, wounds and sputum as stated by [40] and [41]. [42] reported that *Bacillus cereus* is merging as a causative agent for nosocomial infections such as postoperative and posttraumatic wound infections and burns. With a minimum inhibition concentration of 0.5mg/mL as indicated in Table II, this justifies the use of the plant as bandages and treatment of rashes, boils and swelling of the body [7] and [8]. *Staphylococcus epidermidis* is known to cause hospital acquired infections found on the human skin [43]. [44] reported that *S. epidermidis* was isolated from acne vulgaris-affected skin. The bulb extract had inhibited *Staphylococcus epidermidis* at a concentration of 0.0625mg/mL. This supports the traditional use of the bulb for the treatment of itchy rashes, boils and acne. [45] reported that no inhibition was observed for *Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* from the water and methanol crude bulb extract (1mg/mL) for the disc diffusion method. From the experiment done, different results were generated for the 50:50 methanol: dichloromethane crude bulb extract. *Staphylococcus aureus* had an inhibition of 10.67mm, *Staphylococcus epidermidis* 2.67mm and *Bacillus subtilis* 12.67mm, this is evident that the methanol and dichloromethane had extracted more compounds which had more antibacterial activity as compared to the methanol or water crude extract. The same applies to the traditional preparation on the bulb extract, where water is used and as stated by [45], the water extract lacked compounds possessing antibacterial activity as compared to the methanol: dichloromethane extract. Water is used as an extraction solvent by traditional healers since its naturally occurring liquid on Earth, and its chemical and physical properties largely change when varying the temperature and pressure, the polarity of the water is known to be close to that of alcohols [36], [46]. The traditional preparation of medicinal plants is either done by mixing the plant parts with hot or cold water, this is used to treat different aliments [47], [48]. However, from Table II, the PHWE extract for both antibacterial methods failed to inhibit the majority of the bacteria which are considered to be pathogenic. To the best of our knowledge, this is the first time results shown in Table II are being discussed since published articles [45] have only tested the antibacterial activity of methanol and water extract.

Even though PHWE extract was able to inhibit some of the bacteria tested, it is not a good solvent for the extraction of bioactive compounds from *C. macowanii* bulbs as compared to the solvent extract used since the miscibility of bioactive compounds differs in extracts and hence there was a decrease in antibacterial activity.

Potassium had the highest value 45.844mg/kg. this chemical element is known to include relief from stroke, blood pressure, heart and kidney disorders [49]. This justifies the traditional use of the bulb for the treatment of urinary tract problems [7], [8]. Iron was 5.662mg/kg and it is vital for metabolic processes such as DNA synthesis and oxygen transport to cells and for treating chronic disorders like renal failure anemia [50]. Ca present in *C. macowanii* might be therapeutic since its known to be responsible for metabolic processes such as cell division and the regulation of cell proliferation [50]. It is also vital in regulating and strengthening bone mass. Cd and Pb are known to be toxic at low concentration and they were detected at 0.126mg/kg and 0.8mg/kg respectively and their World Health Origination WHO permissible limits are 0.3 and 10 mg/kg respectively [50]. Hg was measured at 0.54436mg/kg and its safety limit is 2 µg/kg [50], therefore precautions have to be taken when consuming the plant for medicinal purposes. Mn is needed in small amounts in the body since it's a co-enzyme in antioxidant processes however, extremely high levels above 13mg/kg which is the safety limit of this essential elements can be toxic. High explosion levels of Mn may cause irritation of the lungs which could lead to pneumonia [51]. Nickel is known to promote breast milk production, however, exposure to high levels of Ni than that normally found in water and food has been reported to cause lung disease and affects stomach and kidneys in dogs and rats. Exposure levels of more than 0.1mg/L in drinking water is considered toxic [52]. Na is an essential nutrient that aids in heart performance, nervous system and glucose absorption [53]. Health effects such as hypertension, cardiovascular disease and bone disease are caused by high consumption of sodium [53]. Cr has been shown to cause skin irritation, kidney damage, circulatory and nerve tissues, respiratory problems and nose bleeds. Cr was detected at 5.607mg/kg and the safety limit is 27mg/kg in Cr (VI) [51], [50].

V. Conclusion

The results obtained indicated a number of phytochemicals present which are associated with treating a number of aliments and the crude solvent extract had strong antibacterial activity against a number of the bacteria tested and thus

indicates that the plant possess a potential in the development of phytomedicines. Further pharmacological and toxicological evaluations have to be done to confirm the safety of the plant for clinical applications. From the results in Table III, the metals detected in *C. macowanii* bulbs are within the permissible limits even though further investigation have to be done on the safety and quality of the plant since its collected from the wild and used for medicinal applications.

VI. REFERENCES

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