A comparison of the pharmacological properties of garden cultivated and *muthi* market-sold *Bowiea volubilis*

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**A B S T R A C T**

Biological activities of petroleum ether (PE), dichloromethane (DCM), 70% ethanol (EtOH) and water extracts of Botanical Garden-grown (BG) and *muthi* market-sourced (MM) *Bowiea volubilis* bulbs were compared. bulb extracts were subjected to the microdilution technique using five test organisms for antimicrobial activity and cyclooxygenase (COX-1 and -2) inhibition as well as the Ames test for potential mutagenicity. Overall, both the MM and BG bulb extracts demonstrated a comparatively weak antimicrobial potency. The best minimum inhibitory concentration (MIC: 1.56 mg/ml) was detected in the MM bulb water extract against *Candida albicans*. In both MM and BG bulbs, 63% of the extracts, particularly the non-polar solvent extracts, exhibited a high (>70% inhibition) COX-1 and -2 inhibitory activity. Both MM and BG bulb extracts were not mutagenic against the *Salmonella typhimurium* TA98 tester strain. Current findings indicate the potential substitution of cultivated *B. volubilis* bulbs (BG) for the wild population (MM) which is often utilized and preferred in traditional medicine. Inevitably, this will contribute to the conservation of the species as the strain on the wild population due to overharvesting will be alleviated.

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1. Introduction

*Bowiea volubilis* Harv. Ex Hook. f., known as *igibisila* in isiZulu is a member of the family Hyacinthaceae and is widely distributed in the eastern part of South Africa. *B. volubilis* is a perennial bulb that grows up to 15 cm in diameter with the bulb half buried in the ground. The tuberous bulb is greenish-white in colour without fibrous outer scales (Van Wyk et al., 2009). The leaves are lanceolate with small greenish flowers that bloom in Spring producing an unpleasant smell (Van Wyk et al., 2009).

In traditional medicine, *B. volubilis* ranks amongst the top 14% of the most traded medicinal plants in South Africa (Mander, 1998). The bulbs contain several active cardiac glycosides such as bovogenin A and the structurally related bufadienolides. Generally, cardiac glycosides increase the force of heart muscle contractions thus constituting therapeutic benefits in cases of congestive heart failure (Page, 1964). However, the consumption of toxic cardiac glycosides can affect the cardiac rhythm and cause disturbances of atrio-ventricular conduction, including a complete atrio-ventricular block (Dai et al., 2011). Nevertheless, *B. volubilis* bulbs are the most commonly used part of the species. Owing to over-harvesting, *B. volubilis* is currently classified as a vulnerable species in the southern African Plant Red Data List (Raimondo et al., 2009). The over-exploitation and harvesting of several medicinal plants have thus resulted in these plants becoming endangered and/or extinct (Fennell et al., 2004). Consequently, researchers have proposed some practical approaches to counteract the risk of extinction (Makunga et al., 2008). For instance, Van Staden (1999) postulated that “small-scale farming” remains a sustainable means for medicinal plant conservation. Nevertheless, the absence of convincing evidence on the potency of cultivated medicinal plants remains a physiological bottleneck. Traditional healers believe that medicinal plants collected from the wild are more potent than the cultivated ones (Cunningham, 1993). Globally, there is an increasing interest in the number of studies focusing on the possible variations or similarities between wild and cultivated medicinal plants (Szőke et al., 2004; Vogel et al., 2011;
Inngjerdingen et al., 2012; Rokaya et al., 2012; Soriano-Melgar et al., 2012). The current study aimed at evaluating the suitability of cultivated *B. volubilis* bulbs as an alternative to wild/natural *muthi* market-sourced bulbs in terms of their biological activities and safety.

2. Materials and methods

2.1. Plant collection and extraction

Bulbs of *B. volubilis* (approximately 5-years-old) were collected in March 2011 from the University of KwaZulu-Natal (UKZN) Botanical Garden and designated as Garden-grown (BG). *Muthi* market-purchased (MM) *B. volubilis* bulbs were obtained from the Pietermaritzburg commercial herbal market, South Africa. The plant was identified by Dr C. Potgieter after which a voucher specimen (Masondo 01) was prepared and deposited at the Bews Herbarium, UKZN, Pietermaritzburg, South Africa.

Both BG and MM bulbs were oven-dried at 50 °C for four days and ground into fine powders using a Culatti Grinder (Janke and Kunkel GMGH, Staufen, Germany). Ground materials (5 g) were extracted sequentially with 100 ml of petroleum ether (PE), dichloromethane (DCM), 70% ethanol (EtOH) and water in a sonication bath for 1 h. The bulb extracts were filtered through Whatman No. 1 filter paper and concentrated in vacuo at 40 °C using a Buchi rotary evaporator. The concentrated extracts were dried over a stream of cold air and the final weights were recorded. Resultant yields (% of the different extracts are shown in Table 1. For antibacterial, antifungal, and anti-inflammatory assays and the Ames test, the organic solvent and water extracts were suspended in EtOH (70%) and water, respectively.

2.2. Biological activity assays

Four bacterial strains (*Bacillus subtilis* ATCC 6051; *Staphylococcus aureus* ATCC 12600; *Escherichia coli* ATCC 11775; *Klebsiella pneumoniae* ATCC 13883) and a fungal strain (*Candida albicans* ATCC 10231) were used for the study. The minimum inhibitory concentration (MIC) of the bulb extracts was evaluated using the antibacterial microdilution technique according to Elloff (1998) and modification for antifungal activity (Masoko et al., 2007). The details of the assays, controls used and measurement of MICs were as outlined by Aremu et al. (2010).

Anti-inflammatory potential was evaluated using cyclooxygenase-1 and -2 (COX-1 and -2) inhibitory assays as described by Jäger et al. (1996) and Zschocke and Van Staden (2000), respectively. We used the same procedures as outlined by Aremu et al. (2010).

The Ames test was used to evaluate the mutagenic effect of the extracts (Maron and Ames, 1983; Mortelmans and Zeiger, 2000). Extracts were re-dissolved in EtOH (70%) to obtain three concentrations of 5, 0.5 and 0.05 mg/ml respectively. The assay conducted without S9 metabolic activation followed the outlines by Ndhlala et al. (2010).

2.3. Data analysis

For reliable results, the assays were conducted twice with either two replicates (antimicrobial and anti-inflammatory assays) or three plates (Ames test) at any particular time. The mean ± standard error of the results was calculated using Graph Pad Prism (version 4.0) statistical software Programme for Windows (GraphPad software Inc.).

3. Results and discussion

3.1. Antimicrobial effect

The MICs of both BG and MM *B. volubilis* bulb extracts were greater than 1 mg/ml (Table 1). The best MIC (1.56 mg/ml) was detected with the MM bulb water extract against *C. albicans*. A total of 25% BG and 45% MM bulb extracts had MICs greater than 10 mg/ml. Amongst the non-polar solvent (PE and DCM) extracts, BG-obtained bulbs had similar or better antimicrobial activity compared to the MM bulbs. On the other hand, the majority of the EtOH and water extracts of MM bulbs were more potent than the BG bulbs. As highlighted by Bairu et al. (2011), the ecotype, age, size and season are some of the factors which may significantly affect the phytochemical and pharmacological activities of plant species. Along this line, it was demonstrated that domestication processes and cultivation conditions increased the antioxidant properties of *Turnera diffusa* (Soriano-Melgar et al., 2012). Therefore, cultivation of medicinal plants can relieve harvest pressure and help meet the increasing demand for such plants. In the current study, the relative similarity in MIC value between the BG and MM indicates the potential of cultivated *B. volubilis* in traditional medicine.

Although the plant is reported to be used for various infections, both BG and MM bulb extracts had MIC values above 1 mg/ml in the current study. This rather poor antimicrobial activity has been demonstrated in previous studies by Stafford et al. (2005) and Buwa and Van Staden (2006). In view of the fact that infectious diseases are caused by a wide variety of microorganisms, it is possible that *B. volubilis* extracts will be effective against other microorganism not tested in the current study.

3.2. Anti-inflammatory effect

Based on a scheme devised by Tunón et al. (1995), four levels of inhibitory activity are defined: below 20% = “insignificant”, between 20 and 40% = “low” activity, from 40 to 70% = “moderate” activity, and above 70% = “high” inhibition. Several studies have shown that medicinal plants potentially provide a useful source of new effective anti-inflammatory agents (Taylor et al., 2001). In both MM and BG bulbs, the majority (63%) of the extracts particularly, the non-polar solvent extract exhibited a high (>70% inhibition) COX-1 and -2 inhibitory activity (Fig. 1). In terms of COX-1 inhibition, more (75%) extracts will be effective against other microorganism not tested in the current study.

### Table 1

| Extract               | Yield (%) | Minimum inhibitory concentration (mg/ml) |
|-----------------------|-----------|-----------------------------------------|
|                       | BG        | MM                                      |
| **Bacillus subtilis** | 0.348     | 0.386                                   |
| **Staphylococcus aureus** | 6.25     | 6.25                                    |
| **Klebsiella pneumoniae** | 6.25     | 12.50                                   |
| **Escherichia coli**  | 6.25      | 6.25                                    |
| **Candida albicans**  | 12.50     | >12.50                                  |
| **DCM**               | 0.716     | 1.040                                   |
|                       | 6.25      | 12.50                                   |
|                       | 3.13      | 6.25                                    |
| **EtOH**              | 4.332     | 4.934                                   |
|                       | 6.25      | 6.25                                    |
|                       | 6.25      | 3.13                                    |
| **Water**             | 1.686     | 3.726                                   |
|                       | 6.52      | 12.50                                   |
|                       | >12.50    | >12.50                                  |
| **Neomycin**          | 1.6 × 10^{-3} | 0.8 × 10^{-3}                  |
| **Amphotericin B**    | 1.6 × 10^{-3} | 0.8 × 10^{-3}                  |
|                       | 9.8 × 10^{-3} |                                          |

PE: Petroleum ether, EtOH: Ethanol, DCM: Dichloromethane, BG: Botanical Garden-grown and MM: Muthi market-sourced bulb.
extracts of BG origin demonstrated a higher percentage inhibition than the MM (50%) extract. Conversely for COX-2 inhibition, MM (75%) extracts were more potent than the BG (50%) extracts. Water extracts of both MM and BG bulb had an insignificant inhibitory activity against COX-2 enzymes. Nevertheless, the MM water extract had about 7-fold better COX-1 inhibitory activity than the BG bulbs. Current findings are in agreement with other studies where it was demonstrated that non-polar solvent extracts of several South African medicinal plants generally possess better COX inhibition than the water extracts (Jäger et al., 1996; Aremu et al., 2010; Bairu et al., 2011). The better inhibitory activity of the non-polar extracts could be due to better extraction of the active principles by the extracting organic solvents (Jäger et al., 1996).

In recent times, focus has been on the production of non-steroidal anti-inflammatory drugs (NSAIDs) which can inhibit COX-2 without having any adverse effect on COX-1 (Fennell et al., 2004). In addition, such drugs/compounds have significant therapeutic value because they are non-ulcerogenic and have anti-inflammatory activity (Mantri and Wittak, 1994). In MM EtOH extract, there was a higher COX-2 inhibition than COX-1 which is an indication that the plant extract is of better pharmacological potential (Fennell et al., 2004). Generally, plants with such COX-2 inhibitory activity are preferred in the search for novel chemicals (Taylor and Van Staden, 2001). Although pain is known to be caused by several physiological pathways, the inhibition of COX-1 and -2 by the extracts (mainly non-polar MM and BG) is further evidence of the efficacy of *B. volubilis* against pain and inflammation-related ailments in traditional medicine (Hutchings et al., 1996). Nevertheless, the effectiveness of both BG and MM *B. volubilis* extracts against other enzymes, for example lipoxygenase (LOX) involved in the LOX pathway may be necessary.

### 3.3. Mutagenic effect

In terms of the number of revertants produced, there were some differences between the MM and BG extracts (Table 2). An estimated 50% of the MM bulb extracts had a greater number of revertants than the BG-grown bulbs. For instance, the number of revertants produced in water extracts of MM bulbs at 0.5 mg/ml was 3-fold higher than that of BG extracts. Overall, all the extracts of the MM and BG bulbs were not mutagenic towards the *Salmonella typhimurium* TA98 tester strain under the investigated conditions. Furthermore, bacterial toxicity was evaluated by observing the background lawn of bacterial growth. The presence of a granular thin film layer on the background lawn indicates the absence of toxicity (Mortelmans and Zeiger, 2000). There was no toxicity detected in both MM and BG extracts as well as in the controls against the *S. typhimurium* tester strain. However, at this stage, the absence of any toxic or mutagenic effects remains preliminary safety findings. Further studies involving the use of more bacterial strains as well as the mimicking of metabolic activation systems of the body will be required for a more reliable verdict.

### 4. Conclusions

For conservation of medicinal plants, the potential of the use of tissue-cultured and cultivated plantlets has been demonstrated for species such as *Tulbaghia violacea* (Ncube et al., 2011) and *Harpagophytum procumbens* (Bairu et al., 2011). From the current study, there was generally no wide variation in the biological activities between MM and BG extracts. It gives an indication that cultivated *B. volubilis* can be used in traditional medicine rather than relying on bulbs from wild populations. In order to meet the increasing

![Fig. 1](https://example.com/fig1.png) Anti-inflammatory activity of Botanical Garden-grown and *muti* market-sourced *Bowiea volubilis* bulb extracts. (A) Percentage COX-1 enzyme inhibition and (B) percentage COX-2 enzyme inhibition. C = control (Indomethacin), PE = petroleum ether, DCM = dichloromethane, EtOH = ethanol, W = water.
Table 2

| Extract                  | Concentration (mg/ml) | Number of His\(^{+}\) revertants |
|-------------------------|-----------------------|----------------------------------|
| Petroleum ether         | 0.05                  | 18.5 ± 4.95 21.0 ± 0.71          |
|                         | 0.05                  | 14.0 ± 7.08 21.0 ± 0.71          |
| Dichloromethane         | 0.05                  | 14.0 ± 7.08 21.0 ± 0.71          |
|                         | 0.05                  | 15.0 ± 4.24 15.0 ± 0.71          |
|                         | 0.05                  | 24.0 ± 4.24 16.2 ± 2.12          |
| Water                   | 0.05                  | 24.0 ± 4.24 16.5 ± 4.95          |
|                         | 0.05                  | 18.5 ± 4.95 18.5 ± 4.95          |
| 4-Nitroquinoline-N-oxide| 0.05                  | 18.5 ± 4.95 18.5 ± 4.95          |

The values are presented as mean ± standard error.

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