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Qualitative and quantitative phytochemical screening and antioxidant potential of *Bulbine inflata* (Asphodelaceae)

Rebecca Opeyemi Oyerinde* and Ida Masana Risenga
School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa

Abstract: *Bulbine inflata* is one of the species in the genus *Bulbine* that are yet to be documented for potential medicinal uses. Hence, we carried out its preliminary phytochemical profiling and investigated its antioxidant potential. The leaves were dried using air- and freeze-drying techniques and were extracted by water, methanol, ethyl acetate and hexane. Various common colour tests were used for the presence of phytochemicals. Some of the phytochemicals were further quantified. Phosphomolybdate, 2, 2 diphenyl-1-picryhydrazyl, hydrogen peroxide and metal chelating assays were used to assess the antioxidant potential of *B. inflata*. Tannin, flavonoids, phenols, glycosides, steroids, coumarins, quinones, saponins and terpenoids were detected phytochemicals in *B. inflata* leaves. The highest total phenolic, flavonoid and tannin contents, as well as total antioxidant capacity, were recorded for water extract. *B. inflata* showed moderate to high antioxidant activities against DPPH, H_2O_2 and metal chelating. Freeze-dried samples presented with higher results than air-dried samples in most assays. The results showed the potential of *B. inflata* for medicinal uses and could expand the ethnomedicinal resources in the communities where it is prevalent and beyond.

Keywords: Antioxidant activities, *Bulbine* species, drying methods, medicinal plants, secondary metabolites, solvents.

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INTRODUCTION

Medicinal plant species have been shown to have curative and preventive effects against human ailments (Sofowora *et al.*, 2013; Prasad, 2016) because of their antioxidant, anticancer, anti-inflammatory, antiseptic and antimicrobial, properties (Aye *et al.*, 2019). Their associated positive effects on humans are attributed to the presence of phytochemicals/plant metabolites, which the plants produce primarily in response to biotic and abiotic stresses (Kumar *et al.*, 2023). The health benefits associated with the presence of biologically active phytochemicals in plants offer great possibilities for their medicinal use and drug discovery (Sasidharan *et al.*, 2011; Koche *et al.*, 2016). Broadly, these phytochemicals are categorised as alkaloids, phenols, terpenoids and steroids and other chemicals (Koche *et al.*, 2016). Each of these categories further comprises subgroups of phytochemicals.

South Africa is one of the countries in the world that are richly endowed with a wide range of plant biodiversity (le Roux *et al.*, 2017), and it is listed in the top five countries of the world with the highest percentage and number of medicinal plants (Chen *et al.*, 2016). South Africa and other Southern African countries are home to a substantial number of plant families that have been used for medicinal purposes (Cock and Van Vuuren, 2020); one of which is Asphodelaceae (Fayisa and Mirete, 2022). Asphodelaceae comprises two subfamilies which are Alooideae and Asphodeloideae and 16 genera (Fayisa and Mirete, 2022). Some of the genera, such as *Aloe*, *Kniphofia* and *Bulbine*, are well known for medicinal and pharmacological uses.

The genus *Bulbine* Wolf is so named because most of the species in the genus have 'bulb-like' tuberous roots. They are also succulent and perennials. The flowers are usually yellow with hairy filaments. The species in the genus are adapted to a wide range of habitats for efficient water use (Williamson and Baijnath, 1995; Naidoo *et al.*, 2011; Bodede and Prinsloo, 2020). There are 78 approved species in the genus (Bodede and Prinsloo, 2020), most of which are indigenous and distributed across Southern Africa while about six are found in Australia (Naidoo *et al.*, 2011; Bodede and Prinsloo, 2020; Gilbert, 2023). However, only 12, out of the 78 approved species, have been documented for medicinal properties and uses. Some of which are *B. frutescens*, *B. abyssinica*, *B. natalensis*, *B. latifolia*,and *B.bulbosa* (Bodede and Prinsloo, 2020). Species in this genus are useful for treating a wide range of health issues, which are majorly skin problems, rheumatism, fertility issues, gastrointestinal problems, sexually transmitted diseases and urinary tract infections (Coopoosamy, 2011; Bodede and Prinsloo, 2020).

B. inflata is one of the *Bulbine* species that has no documented information for phytochemical screening and medicinal properties. In addition to the general characteristics of the species in the genus, *B. inflata* has inflated fruits (fig. 1) as its distinguishing trait, which suggests the species name '*inflata*'.

Considering that about 80% of South African and other developing countries of the world directly or indirectly

**Corresponding author:* e-mail: rebecca.oyerinde@wits.ac.za

use medicinal plants and their products (Chen *et al.*, 2016; Volenzo and Odiyo, 2020) and that the increase in the world's population has a proportional increase in the demand for medicinal plants (Chen *et al.*, 2016), it is important to have knowledge of a wide range of plants that have medicinal properties rather than depending solely on those that are known and established. *B. inflata* may be another species with the potential for medicinal use just like other species in the genus. The aim of this work, therefore, was to carry out preliminary screening on some phytochemical constituents and antioxidant activities of *B. inflata*.

MATERIALS AND METHODS

All the reagents and chemicals that were used in this study were of analytical grades and they were bought from Sigma-Aldrich, USA. All absorbance readings were taken with a Thermo-Fisher Scientific Genesys (GEN10S UV-VIS) spectrophotometer.

Plant material collection, preparation, extraction and yield

The leaves of *B. inflata* (fig. 1) were harvested from the University of the Witwatersrand (26.1907°S, 28.0314°E), Johannesburg, South Africa in 2023. The species was identified by Dr Ida Risenga and the voucher specimen (IR01) was deposited at the herbarium of the same University. The leaves were cleaned and one half was air-dried in the oven (Binder oven) at 33±2°C while the other half was freeze-dried (Zirbus technology freeze-dryer) at -83°C, both for 3 days. The dried leaves were subsequently ground to fine powder.

Water and methanol as the polar solvents and ethyl acetate and hexane as the non-polar solvents, were used for the crude extraction of phytochemicals from the powdered leaves. Three grams of leaf powder was added to 25 ml of each solvent, agitated for 72 hrs in an orbital shaker at 150 rpm and subsequently centrifuged for 5 minutes at 3500 rpm. The supernatants, which served as the crude extracts, were decanted and kept in the refrigerator for this research while the residues were discarded. The percentage yield of extracted sample was calculated as:

% yield = (dry extract (without solvent) ÷ weight of ground sample) × 100

Qualitative phytochemical screening

Various colour tests for alkaloids, tannins, saponins, flavonoids, glycosides, steroids, volatile oils, coumarins, phlobatannins, quinones, terpenoids, cardiac glycosides and phenolics were carried out using standard published procedures (Gul *et al.*, 2017; Tyagi, 2017; Roghini and Vijayalakshmi, 2018; Akinyemi and Oyelere, 2019; Shaikh and Patil, 2020; Teffo *et al.*, 2022).

Quantitative phytochemical screening

Total flavonoid content (TFC)

Four millilitre (4 ml) of distilled water was added to 0.3 ml of crude extract. Then, 0.3 ml of 5% sodium nitrate ($NaNO_3$) was added, mixed thoroughly and allowed to rest for 5 minutes. Thereafter, 3 ml of 10% aluminium chloride ($AlCl_3$) solution was added and left for 6 minutes, after which 2 ml of 1M NaOH solution was added. The mixture was made up to 10 ml by the addition of 0.4 ml of distilled water and then incubated for 1 hr in the dark at room temperature. The absorbance of the solution was measured at 510 nm wavelength and 80% methanol was used as the blank. A linear equation generated from quercetin standard calibration curve was used to calculate the TFC:

$y = 0.2388x - 0.0019$ ($r^2 = 0.9997$)

Total phenolic content (TPC)

TPC was determined by the Folin-Ciocalteu (FC) reagent assay. The FC reagent was diluted to 10% strength before its use. For each crude extract, 2 ml of 7.5% sodium carbonate (Na_2CO_3) was added to 0.2 ml of extract. This was followed by the addition of 0.75 ml of diluted FC reagent and 7 ml of distilled water. The solution was incubated for 2 hrs in the dark at room temperature. The absorbance of the solution was measured at 765 nm wavelength and 80% methanol was used as the blank. A linear equation generated from gallic acid standard calibration curve was used to calculate the TPC:

$y = 0.0495x - 0.0259$ ($r^2 = 0.9994$)

Total tannin content (TTC)

The TTC from the crude extract of *B. inflata* leaves was determined as follows: 0.1 ml of crude extract was added to 7.5 ml of distilled water. Thereafter, 0.5 ml of undiluted FC reagent was added, which was followed by the addition of 1 ml of 35% sodium carbonate (Na_2CO_3). The volume was made up to 10 ml by the addition of 0.9 ml of distilled water. The solution was incubated at room temperature in the dark for 30 minutes. The absorbance of the solution was measured at a wavelength of 725 nm (Lahare *et al.*, 2021; Teffo *et al.*, 2022). A linear equation generated from gallic acid standard calibration curve was used to calculate the TTC:

$y = 0.046x - 0.0264$ ($r^2 = 0.9833$)

Total proanthocyanidin content (TPAC)

The vanillin-methanol method was adopted for the determination of TPAC (Oyedemi *et al.*, 2010). Crude extract (0.5 ml) was added to 3 ml of 4% vanillin-methanol solution, which was followed by the addition of 1.5 ml of HCl. The mixture was mixed vigorously and incubated for 15 minutes in the dark at room temperature. The absorbance of the solution was measured at a

wavelength of 500 nm. A linear equation generated from catechin calibration curve was used to calculate the TPAC:

$y = 0.9554x + 0.0003$ ($r^2 = 0.9927$)

In vitro antioxidant activity assays

Total antioxidant capacity by phosphomolybdate method and three antioxidant assays, i.e. 2, 2 diphenyl-1-picryhydrazyl (DPPH) scavenging activity assay, hydrogen peroxide (H_2O_2) assay and metal chelating assay, were investigated in this study. All assays were carried out using various volumes (10, 20, 30, 40 and 50 µl) of crude extracts in capped test tubes. Each assay was done in triplicate. After the required incubation period for each assay, the solutions were dispensed into cuvettes and the spectrophotometer earlier described was used to measure the absorbances at required wavelengths. The antioxidant activity percentage of *B. inflata* leaf extract, against DPPH and H_2O_2 radicals, was calculated as:

$$\% \text{ activity} = ([A_0 - A_1]/ A_0) \times 100$$

A_0 and A_1 represented the absorbance of the control and the extracted sample, respectively. A linear graph equation generated from the various volumes against the percentage activity was used to calculate the IC_{50} for DPPH and H_2O_2. IC_{50} is the half-maximal inhibitory concentration of the extract required to inhibit the oxidative activity of the tested radicals by 50%.

Total antioxidant capacity (TAC) by phosphomolybdate method

The TAC of *B. inflata* leaves was measured using phosphomolybdate reagent and ascorbic acid as the standard. A volume of 0.1 ml of the crude extract for each solvent and each drying method was added to 1 ml of the reagent (made by combining equal volumes of 28 mM sodium phosphate, 0.6 M H_2SO_4 and 4 mM ammonium molybdate) in capped tubes. The solutions were heated in a water bath at 95˚C for 90 minutes. The solutions were left to cool completely after which the absorbance was measured at 695 nm wavelength against 80% methanol as blank (Prieto *et al.*, 1999). A linear equation generated from ascorbic acid calibration curve was used to calculate the TAC:

$y = 0.0036x + 0.1161$ ($r^2 = 0.9985$)

Diphenyl-1-picryhydrazyl (DPPH) scavenging activity assay

A stock solution of DPPH was made by dissolving 50 mg of DPPH in 100 ml of 80% methanol. DPPH work solution was prepared by mixing one part of the stock solution with four parts of 80% methanol. For the scavenging of DPPH by the crude extract of *B. inflata* leaves, 700 µl of the work solution was added to various volumes (as above) of extract. The final volume was made up to 1 ml by the addition of respective solvents. The reaction was allowed to take place in the dark for 45 minutes at room temperature. The absorbance of the mixture was measured at a wavelength of 517 nm. The control reaction was made up of 700 µl of DPPH work solution mixed with 300 µl of solvent (without the extract) while 80% methanol was used for the blank (Teffo *et al.*, 2022). The percentage scavenging of DPPH and IC_{50} were calculated as earlier described.

Hydrogen peroxide (H_2O_2) assay

A 40 mM solution of H_2O_2 was prepared by mixing phosphate buffer and 30% H_2O_2. Thereafter, 600 µl of 40 mM H_2O_2 solution was added to various volumes (as stated earlier) of crude extract. The volume was made up to 1 ml by the solvent used for the extraction. The solution was incubated for 10 minutes in the dark and at room temperature, after which the absorbance was measured at a wavelength of 230 nm. The phosphate buffer, without the H_2O_2, was used as the blank, while 600 µl of 40 mM H_2O_2 solution combined with 400 µl of solvent (without the extract) was used as the control (Teffo *et al.*, 2022). The percentage scavenging of H_2O_2 by *B. inflata* and IC_{50} was calculated as earlier described.

Metal chelating assay

Various volumes (specified earlier) of *B. inflata* leaf crude extracts were made up to 1 ml with the various solvents used for extraction. Thereafter, 50 µl of 2 mM $FeCl_3$ was added to the 1 ml mixture (of extract and solvent) and shaken vigorously with a vortex. Subsequently, 200 µl of 5 mM ferrozine was added and the mixture was shaken again. The mixture was then incubated in the dark and at room temperature for 10 minutes and the absorbance was taken at a wavelength of 562 nm. 1 ml of solvent mixed with 50 µl of 2 mM $FeCl_3$ and 200 µl of 5 mM Ferrozine served as control while 80% methanol was used as the blank (Pavithra and Vadivukkarasi, 2015; Rakesh *et al.*, 2021). The difference between the absorbance of samples and the control was taken as the actual sample absorbance.

STATISTICAL ANALYSIS

Values obtained from quantitative phytochemical screening assays and *in vitro* antioxidant assays were carried out in triplicates. Pearson Correlation Coefficient (r) was used to assess the relationship between Total Antioxidant Capacity (TAC) and quantified phytochemicals. R Studio (R 4.2.3) was used to analyse all collected data. The obtained values were expressed as mean ± standard deviation (SD). One-way Analysis of variance (ANOVA) was used to determine significant differences at $P \leq 0.05$. Thereafter, Tukey HSD posthoc test was used to determine where the significant differences lie.

RESULTS

Yield

The percentage of extraction yields, based on solvents and drying methods of *B. inflata* leaves, are shown in fig. 2. Methanol and water, which are the polar solvents, gave more extraction yield in comparison with the non-polar solvents, which are hexane and ethyl acetate. Except for methanolic extract, the yields of freeze-dried samples were slightly higher than those obtained from air-dried samples. The highest yield was in the water extract of freeze-dried sample at 37.11% while the lowest yield was in the hexane extract of air-dried sample at 7.77%. The order of yield percentage according to solvents was (water, methanol, ethyl acetate and hexane (fig. 2).

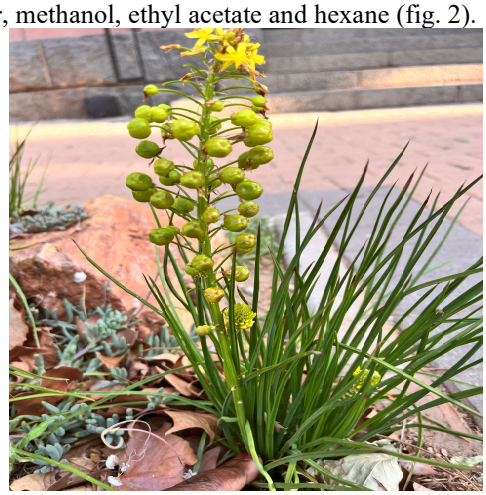

Fig. 1: *B. inflata* showing the leaves, fruits and flowers.

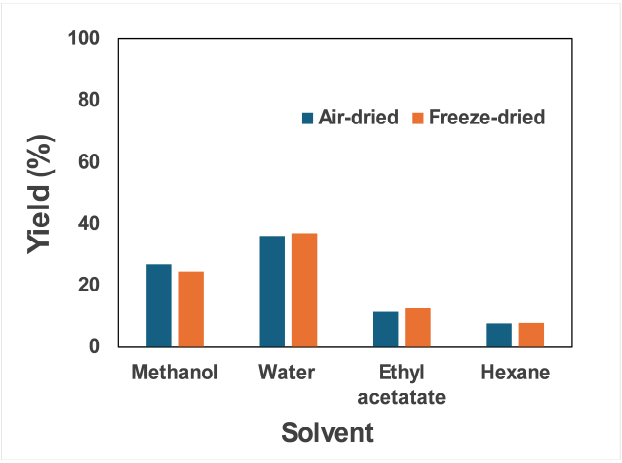

Fig. 2: The yield percentage of *B. inflata* leaf extracts using various solvents and two drying methods.

Qualitative phytochemical screening

The detection of some secondary metabolites such as phenolics, tannins, terpenoids, glycosides, flavonoids among others, in the powdered dried leaves of *B. inflata* was presented in table 1. Most tested phytochemicals were detected in the extracts of polar solvents, especially in water, while extracts of ethyl acetate gave the least indication of the presence of the phytochemicals. Tannin was detected in all extracts of used solvents and in both drying methods.

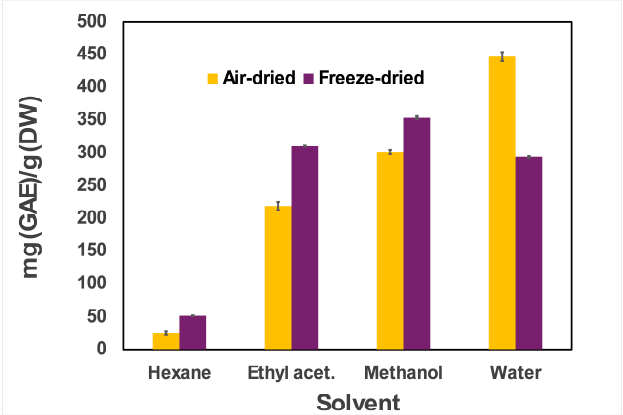

Fig. 3: Total phenolic content (TPC) of *B. inflata*, dried under two conditions and extracted with four different solvents. Bars represented mean values with standard deviation (SD) and different alphabets implied significant difference ($P \leq 0.05$).

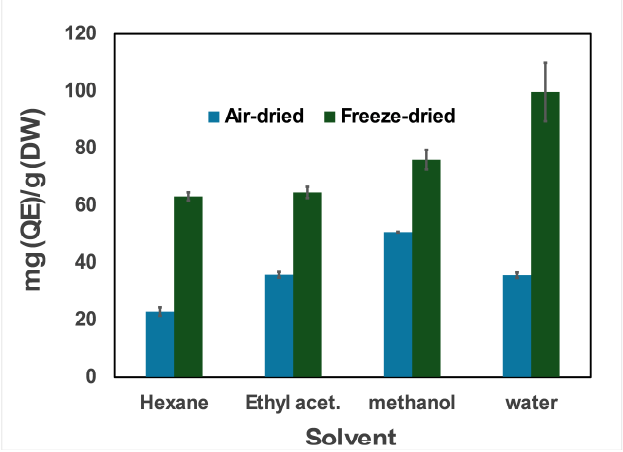

Fig. 4: Total flavonoid content (TFC) of *B. inflata*, dried under two conditions and extracted with four different solvents. Bars represented mean values with SD and different alphabets implied significant difference ($P \leq 0.05$).

Phenolics were detected in both drying methods and all tested solvents, except for hexane. Saponin and phlobatannin were found only in the methanolic extracts. Quinones were detected only in the polar solvent extracts of freeze-dried samples. As indicated in the percentage yield, most of the phytochemicals were detected in polar solvents and freeze-dried samples in comparison with non-polar solvents and air-dried samples (table 1).

Quantitative phytochemical screening

Total phenolic content (TPC)

Ground dried leaves of *B. inflata* showed high total phenolic content (TPC) as shown in fig. 3. Three out of

the four solvents were able to extract between 218-446 mg gallic acid equivalent per g dry weight (mg GAE/g DW) of *B. inflata* leaf sample. Hexane was the only solvent that did not extract a relatively high amount of TPC (<60 mg GAE/g DW). Air-dried water extract significantly ($p \leq 0.05$) had the highest TPC value (446.56 mg GAE/g DW), while air-dried hexane extract had the least TPC content (25.40 mg GAE/g DW). Except in water extract, all freeze dried samples had higher TPC than the air-dried samples.

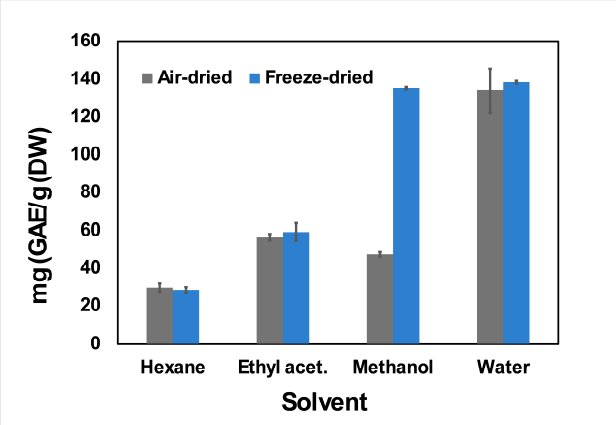

Fig. 5: Total tannin content (TTC) of *B. inflata*, dried under two conditions and extracted with four different solvents. Bars represented mean values with SD and different alphabets implied significant difference ($P \leq 0.05$).

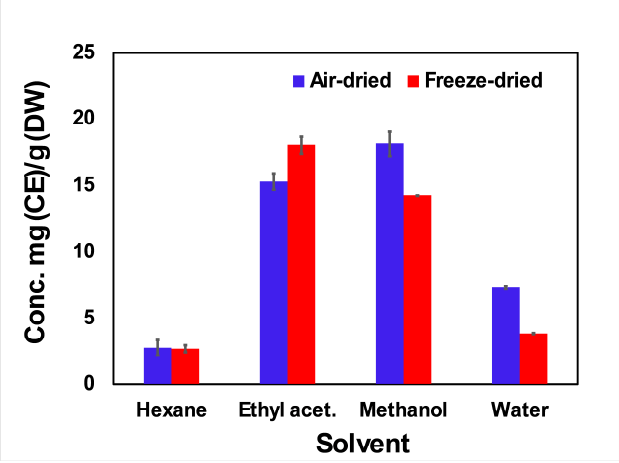

Fig. 6: Total proanthocyanidin content (TPAC) of *B. inflata*, dried under two conditions and extracted with four different solvents. Bars represented mean values with SD and different alphabets implied significant difference ($P \leq 0.05$).

Total flavonoid content (TFC)

Total flavonoid content (TFC) in *B. inflata* leaves was significantly ($p \leq 0.05$) the highest in freeze-dried water extracted sample (99.75±10.29 mg quercetin equivalent (QE)/g DW) while the least was recorded in air-dried hexane extract (22.95±1.41 mg QE/g DW). For each solvent, TFC in freeze-dried samples were significantly higher than in air-dried samples (fig. 4).

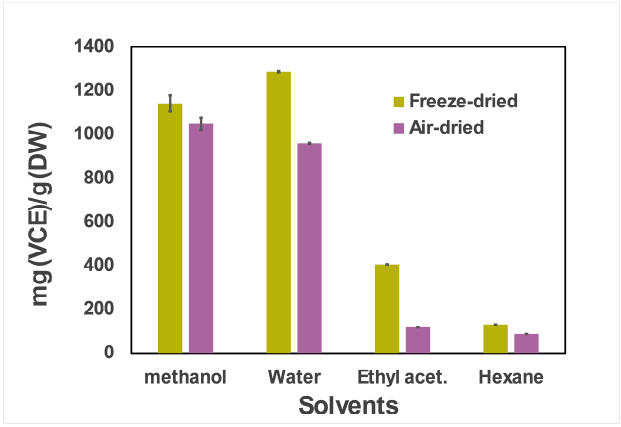

Fig. 7: Total antioxidant capacity/reducing power of *B. inflata*, dried under two conditions and extracted with four different solvents. Bars represented mean values with SD and different alphabets implied significant difference ($P \leq 0.05$).

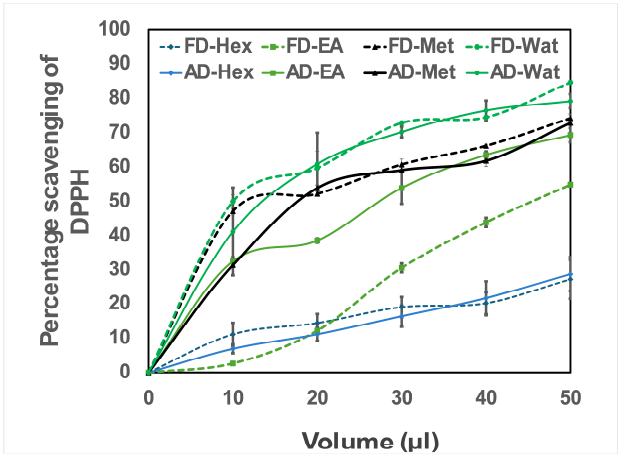

Fig. 8: Scavenging of DPPH by *B. inflata*, dried under two conditions and extracted with four different solvents.

Total tannin content (TTC)

In *B. inflata*, quantitative analysis showed the highest presence of tannins in freeze-dried water and methanol as well as air-dried water extracted samples (138.47±0.72; 135.10±0.84 and 133.89±11.71 mg GAE/g DW, respectively). The least amount of tannin was recorded in the non-polar hexane extracted samples (29.66±2.29 and 28.39±1.52 mg GAE/g DW for air-dried and freeze-dried samples, respectively) (fig. 5).

Total proanthocyanidin content (TPAC)

Total proanthocyanidin content was highest in air-dried methanolic extract of *B. inflata* (18.12±0.91 catechin equivalent (CE)/g DW) and freeze-dried ethyl acetate extract (18.02±0.65 mg CE/g DW) (fig. 6). Surprisingly, the TPAC extracted by water was at its lowest level (fig. 6) in contrast with other tested phytochemicals (figs. 2-4).

In vitro antioxidant activities

Phosphomolybdenum assay for total antioxidant capacity (TAC)

In *B. inflata*, TAC was higher in freeze-dried samples than in the air-dried samples (fig. 7). In addition, samples extracted with polar solvents had higher TAC in comparison with non-polar solvents. Freeze-dried water extract had the highest TAC (1289.79±2.74 mg ascorbic acid equivalent (AAE)/g DW), while air-dried hexane extract had the lowest TAC (87.75±0.28 mg AAE/g DW) (fig. 7). In most cases, The TAC has very strong correlation with quantified phytochemicals, i.e. TPC, TFC, TTC and TPAC (table 2).

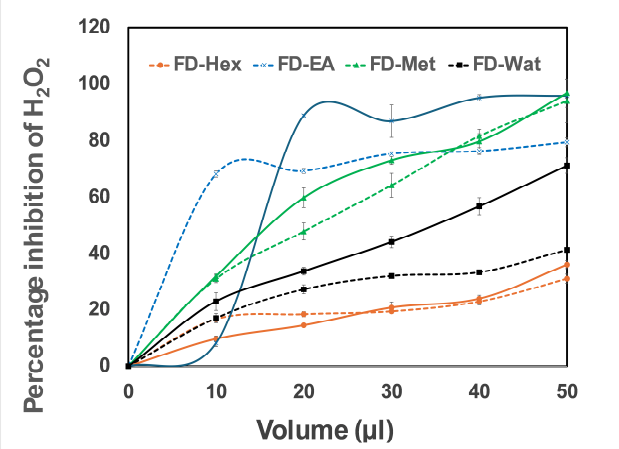

Fig. 9: Inhibition of H_2O_2 by *B. inflata*, dried under two conditions and extracted with four different solvents.

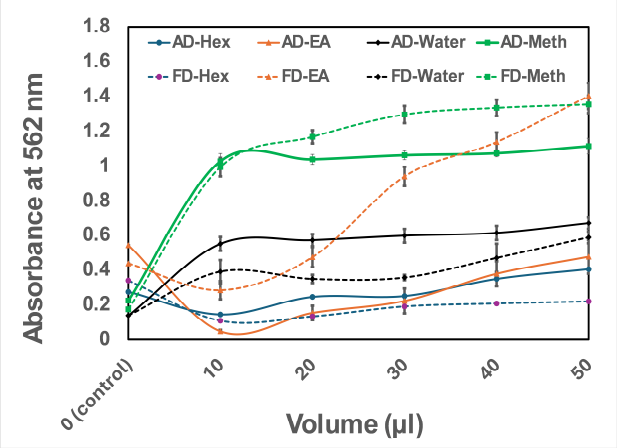

Fig. 10: Metal chelation of iron III ions by *B. inflata*, dried under two conditions and extracted with four different solvents.

Diphenyl-1-picryhydrazil (DPPH) assay

Water extract of *B. inflata* leaves had the highest DPPH inhibition capacity for both freeze-dried (84.6±0.23%) and air-dried (79.18±2.04%) samples (fig. 8). Small concentrations of water extracts of freeze- and air-dried *B. inflata* leaves (0.81±0.22 and 1.22±0.64 mg/ml, respectively) were required to scavenge 50% of DPPH (IC_{50} values, table 3). Samples extracted with non-polar solvents, hexane and ethyl acetate, were not as potent as those extracted with polar solvents i.e., water and methanol. The least inhibition capacities were recorded for hexane extracts at 27.21±5.79% and 28.76±5.08%, for freeze-dried and air-dried samples, respectively (fig. 8), with corresponding IC_{50} values of 13.75±7.21 mg/ml and 9.23±2.59 mg/ml for freeze-dried and air-dried samples, respectively (table 3).

Hydrogen peroxide (H_2O_2) assay

Ethyl acetate extract from air-dried leaves of *B. inflata* had the highest antioxidant activity against H_2O_2, scavenging 95.9% of H_2O_2 (fig. 9) with IC_{50} of 1.98±0.04 mg/ml (table 3). Similarly, methanolic extracts had IC_{50} of 2.50±0.14 and 2.32±0.18 mg/ml, for both air- and freeze-dried samples, respectively (table 3). However, hexane extracts had the least antioxidant capacity just as seen in the other tested parameters (table 3 and fig. 9).

Metal chelating assay

The colour of the Fe-ferrozine complex deepened when extracts of *B. inflata* leaf were added as shown by the absorbance values (fig. 10). Methanolic extracts for both air- and freeze-dried samples as well as ethyl acetate extracts of freeze-dried samples had the highest colour intensity and absorbance values at 50 µl. On the other hand, hexane extract of freeze-dried showed the least colour intensity and absorbance (fig. 10).

DISCUSSION

The concentration of bioactive compounds in plants is usually low, hence, suitable solvents, as well as extraction methods with minimal or no damage to the compounds, are requisites for optimal yield (Dhanani *et al.*, 2017). Higher polarity of solvents have been linked with higher extraction yield (El Mannoubi, 2023). In addition, different drying methods have been shown to affect the yield of bioactive compound extraction (Poorgharib et al. 2023). Among the four solvents that were used in this study, water has the highest polarity while hexane has the least (Adeleye and Risenga, 2022). Thus, the percentage yield of extracted phytochemicals from *B. inflata* was directly proportional to the polarity of solvents (fig. 2). The reduced yield and lack of detection of some phytochemicals could be attributed to (i) The solubility of the bioactive compounds in the solvents as well as the nature of the plant material (Teffo *et al.*, 2022). (ii) The inability of a drying method to effectively disrupt cellular components for optimal bioactive compound release and extraction (Kolawole *et al.*, 2018).

In *B. inflata*, most phytochemicals were detected in the freeze-dried, polar solvent extracted samples. *B. inflata* presented with substantial amount of phenolics and flavonoids (fig. 3 and 4).

Table 1: Colour test for phytochemical screening of *B. inflata* samples.

Phytochemicals	Air-dried				Freeze-dried			
	Hexane	Ethyl acetate	Methanol	water	Hexane	Ethyl acetate	Methanol	water
Saponins	-	-	+	-	-	-	+	-
Terpenoids	-	-	+++	+	+	-	+++	++
Glycosides	-	-	-	+++	-	-	-	+++
Steroids	-	-	+++	+	-	-	++	+++
Volatile oils	+++	-	-	-	++	+	-	-
Coumarins	+	-	-	-	++	-	-	+++
Phlobatannins	-	-	-	-		-	+	-
Alkaloids	-	-	-	-	-	-	-	-
Phenolics	-	++	+++	+++	-	++	+++	++
Tannins								
• Bromine water test	+++	++	++	+	+++	++	++	++
• $FeCl_3$ test	-	-	-	-	-	-	+	-
Quinones	-	-	-	-	-	-	+	+++
Cardiac glycosides	-	-	-	++	-	-	++	-
Flavonoid								
• NaOH test	+++	-	-	-	++	+	-	++
• Conc. H_2SO_4	-	-	-	-	-	-	-	+
• Conc. HCl	-	-	-	-	-	-	-	-

-, +, ++ and +++ represent absence, low, moderate and high detection of phytochemicals, respectively.

Table 2: The Pearson's Correlation Coeffiecient (r) between total antioxidant capacity (TAC) and quantified phytochemicals (TPC, TFC, TTC and TPAC) of *B. inflata* leaves.

TAC	TPC	TFC	TTC	TPAC
AD-Hex	0.984324*	0.804906*	0.631798**	0.372141
FD-Hex	0.654654**	0.066983	0.715554**	0.953821*
AD-EA	0.978883*	0.978450*	0.992336*	0.632381**
FD-EA	0.618590**	0.641053**	0.999424*	0.927247*
AD-Meth	0.081211	0.775506**	0.739186**	0.393159
FD-Meth	0.991799*	0.365263	0.995981*	0.862886*
AD-Water	0.785714**	0.841698*	0.778434**	0.716934**
FD-Water	0.912245*	0.586712***	0.810885*	0.214809

*, ** and *** depict a 'very strong (0.80-1.00), strong (0.60-0.79) and moderate 0.4-0.59)' correlation.

Table 3: Half-maximal inhibitory concentration (IC_{50}) of *B. inflata* on various oxidative molecules.

Drying method/solvent	IC_{50} + SD (mg/ml)	
	DPPH	H_2O_2
AD-Hex	9.23 ± 2.59^{ac}	7.93 ± 0.56^{a}
FD-Hex	13.75 ± 7.21^{c}	14.16 ± 0.97^{b}
AD-EA	2.84 ± 0.174^{ab}	1.98 ± 0.04^{c}
FD-EA	4.55 ± 0.04^{ab}	5.81 ± 0.63^{d}
AD-Meth	2.36 ± 0.07^{ab}	2.50 ± 0.14^{c}
FD-Meth	1.51 ± 0.11^{b}	2.32 ± 0.18^{c}
AD-Water	1.23 ± 0.64^{b}	4.05 ± 0.38^{e}
FD-Water	0.81 ± 0.22^{b}	8.62 ± 0.57^{a}

Different superscripts for each molecule implied significant differences AD: air-dried, FD: freeze-dried, Hex: hexane, EA: ethyl acetate, Meth: methanol.

The phenolic class of phytochemical comprises the largest group of bioactive compounds in plants; while the flavonoids are one of the four groups of phenolics constituting about 66% of phenolic content in dietary plant products (Ozcan *et al.*, 2014; Kumar and Goel, 2019). The presence of the phenol group confers antibacterial, antioxidant, vasodilating and anticancer properties on plants; and makes the plants serve protective roles against mutagenic and degenerative health issues (Ozcan *et al.*, 2014; Koche *et al.*, 2016; Kumar and Goel, 2019). flavonoids have also been reported to have a reducing effect on Type 2 diabetes and cardiovascular diseases (Di Lorenzo *et al.*, 2021). Thus, the consumption of phenolic-rich plant products offer a wide range of benefits to humans (Lin *et al.*, 2016; Rahman *et al.*, 2021).

Tannins, which were detected in *B. inflata* (fig. 5), have antioxidant properties thus, are effective as free radical scavengers. They inhibit the growth of antibiotic-resistant *bacteria* species as well as the ulcer-causing *Helicobacter pylori*. They have also been shown to have antiviral, antidiabetic and anti-inflammatory properties. Thus, they are able to prevent chronic diseases and inflammation-associated ailments (Kibiti and Afolayan, 2015; Sieniawska, 2015). Proanthocyanidins, also known as condensed tannin, were present in *B. inflata* (fig. 6). They are one of the major group of tannin with complex structures, which make them resistant to hydrolysis and are associated with bitter taste of plant products (Sieniawska, 2015; Koche *et al.*, 2016; Singh and Kumar, 2019). They also have the health benefits associated with tannins and polyphenols. These compounds have been detected in other *Bulbine* species such as *B. natalensis* (Yakubu and Afolayan, 2009) and *B. abyssinica* (Kibiti and Afolayan, 2015; Teffo *et al.*, 2022). However, the content of proanthocyanidin was lowest in the water extract of *B. inflata*. This could be attributed to the resistance of proanthocyanidin to hydrolysis unlike the hydrolysable tannin group.

Importantly, all the phytochemicals that were detected in other *Bulbine* species of medicinal importance such as *B. abyssinica* leaves (Teffo *et al.*, 2022), *B. natalensis* stems with the exception of anthraquinones (Yakubu and Afolayan, 2009) and *B. frutescens* (Shikalepo *et al.*, 2018) plants except alkaloids were found in *B. inflata* (table 1). Hence, *B. inflata* may serve medicinal purposes like other members of the family.

Free radicals, such as hydrogen peroxide, are molecules that have unbound/unpaired electrons in their outer layer, which make them unstable and highly reactive. They are formed naturally in human body as byproducts of various physiological and biological activities and a moderate amount of free radicals is essential for human physiological functions. However, when they are in excess, their activities result in oxidative stress, which have degenerative effects that are implicated in various ailments, some of which are lethal (Pham-Huy *et al.*, 2008; Jakubczyk *et al.*, 2020; Munteanu and Apetrei, 2021). Antioxidants are naturally occurring or synthetic compounds that can scavenge and take up the unpaired electrons from free radicals, making them stable and less reactive (Pham-Huy *et al.*, 2008; Gulcin and Alwasel, 2022). While synthetic antioxidants may be more effective, available and stable than their natural counterpart, natural antioxidants are safer (Gulcin and Alwasel, 2022).

The total antioxidant capacity by the phosphomolybdenum assay is based on the ability of antioxidants, such as those that are present in plant extracts, to reduce molybdenum VI (Mo^{VI}) to molybdenum V (Mo^{V}). The reduced Mo^{V} forms a green complex with the phosphate component of the reagent, which shows the highest absorption at 695 nm in the spectrophotometer (Bhatti *et al.*, 2015). The intensity of the green colour is a measure of the reduction from Mo^{VI} to Mo^{V}, which is directly proportional to higher absorbance and the total antioxidant capacity (TAC)/reducing power of the plant extract. With these parameters, *B. inflata*, showed antioxidant and reducing properties, with the freeze-dried water extract presenting the highest TAC. Similarly, aqueous extract of *B. abyssinica* showed the highest TAC in comparison with other solvents (Idamokoro and Afolayan, 2020).

DPPH is a stable free radical molecule that is often used to test and measure the antioxidant capacity of materials such as plant extracts (Kedare and Singh, 2011). The deep purple colour of DPPH reduces when it is mixed with a reducing agent, such as antioxidants. The antioxidants donate electrons, which pair with the unpaired electrons of DPPH, t reducing it to DPPH.H (2,2-diphenyl-1-picrylhydrazine). The degree of discoloration of the DPPH purple colour is a measure of the antioxidant potency of the plant extracts (Kedare and Singh, 2011). Hydrogen peroxide (H_2O_2) is a member of the reactive oxygen species (ROS), which are molecules that are responsible for oxidative stress (Jakubczyk *et al.*, 2020). It is important that there is a balance between their production and elimination by antioxidants, in order to prevent their potential degenerative damage. Medicinal plants, including those in the *Bulbine* genus, have been shown to be highly effective in scavenging these molecules. The IC_{50} for DPPH scavenging by *B. inflata* was within the range of other *Bulbine* species, e.g. 0.0962, 0.1380 and 4.79 mg/ml for the aqueous and methanolic leaf extract of *B. abyssinica*; 0.053 and 0.601 mg/ml for the aqueous methanolic leaf extract of *B. frutescens* and *B. natalensis* (Ghuman *et al.*, 2019; Idamokoro and Afolayan, 2020; Teffo *et al.*, 2022). Examples in the genus *Bulbine* include *B. abyssinica* (Kibiti and Afolayan,

2015; Teffo *et al.*, 2022); *B. frutescens* (Shikalepo *et al.*, 2018; Ghuman *et al.*, 2019) and *B. natalensis* (Ghuman *et al.*, 2019). Similarly, *B. inflata* presented with high antioxidant capacity against these molecules, not only because of their high scavenging percentages, but also because of the IC_{50} values of the extracts.

Essential heavy metals, such as iron, cobalt, copper and zinc, are necessary components for normal physiological and biological functions in humans. However, heavy metals can become toxic when they are accumulated beyond the required threshold in the human systems, causing metal poisoning, oxidative stress and the consequent associated health complications (Gulcin and Alwasel, 2022). Metal chelation is a therapeutic process in which antioxidants are used to inhibit metal toxicity by binding to the metals, thus preventing their harmful effects. Higher affinity for metal binding corresponds to higher antioxidant capacity. Ferrozine has a high binding affinity for iron (Fe) ions and when there is contact between these molecules, an Fe-ferrozine complex is formed immediately (Gulcin and Alwasel, 2022). In this research, ferric ion (Fe^{3+}) was used as opposed to the commonly used ferrous ion (Fe^{2+}) and the complex formed was a light purple/lilac solution. The observed result was opposed to what is reported in literature, that the addition of extracts with antioxidant/chelating properties should prevent or inhibit the formation of Fe-ferrozine complex, leading to a reduction in colour intensity (Adjimani and Asare, 2015; Gulcin and Alwasel, 2022). Instead, the addition of *B. inflata* extracts increased the colour intensity of the solution. The probable explanation was that since *B. inflata* leaf extract has been shown to be a good reducing agent as reported earlier, the Fe (III) ion used for this assay was reduced to Fe (II) ion by the extracts. The Fe (II) ions then bound with the ferrozine to give deeper colour intensity. The second probable explanation was that *B. inflata* leaf was rich in iron. This could be in line with the finding that Fe was one of the mineral components in one of the *Bulbine* species, *B. abyssinica* (Kibiti and Afolayan, 2018). In that regard, the extracts could have released more Fe ions to the solution, leading to more Fe-ferrozine complex formation and the consequent deep colour intensity. This possibility could position *B. inflata* as a remedy for iron-deficient conditions.

CONCLUSIONS

The presence of prominent phytochemicals in *B. inflata*, which are known to confer medicinal attributes to medicinal plants, as well as its high reducing power and antioxidant capacity suggest its potential for use as a medicinal plant. The use of non-polar solvents has no added advantage to the extraction of phytochemicals in *B. inflata*. However, natural drying methods, such as air-drying, presented a safe, cost-effective and easy to use methods without a significant loss in the antioxidant properties of *B. inflata*.

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