Bioactive metabolites of *Bulbine natalensis* (Baker): Isolation, characterization, and antioxidant properties

Olusola Bodede[1,2], Nomfundo Mahlangeni[2], Roshila Moodley[2], Manimbulu Nlooto[1], Elizabeth Ojewole[1]

[1]*Discipline of Pharmaceutical Sciences, School of Health Sciences, University of KwaZulu-Natal, Durban, 4000, KwaZulu-Natal, South Africa,* [2]*School of Chemistry and Physics, University of KwaZulu-Natal, Westville Campus, Private Bag X54001, Durban, 4000, South Africa*

Abstract

Background: Medicinal plants continue to play a key role in disease management and modern drug development. *Bulbine natalensis* is one of several South Africa's indigenous succulent medicinal species. *B. natalensis'* high medicinal profile has made it a commercially-available herb within the South African market and beyond. However, there is a limited scientific report on its bioactive metabolites. **Objectives:** This study's objective was to isolate and identify bioactive compounds from *B. natalensis* leaves and evaluate the compounds and crude extracts for antioxidant activity. **Materials and Methods:** Fractionation and purification of *B. natalensis* dichloromethane extract were done using chromatographic techniques. Whole extract profiling was carried out on dichloromethane and methanol extracts using gas chromatography-mass spectrometry (GC-MS). The isolated compounds and extracts were evaluated for antioxidant activity. **Results:** The dichloromethane extract yielded two pentacyclic triterpenes (glutinol and taraxerol), one tetracyclic triterpene (*β*-sitosterol), a phytylated porphyrin pigment, pheophytin a and knipholone, and a phenyl anthraquinone. GC-MS analysis and ^{1}H nuclear magnetic resonance (^{1}HNMR) fingerprinting of the leaf extracts identified additional bioactive metabolites as *α*- and *β*-tocopherol, campesterol, *p*-hydroxyphenylacetic acid, and long-chain alcohols. The antioxidant assays revealed the methanol extract to have good ferric reducing antioxidant power while dichloromethane extract displayed stronger Mo(IV) reducing power compared to *α*-tocopherol and isolated compounds. **Conclusion:** This study revealed previously unreported compounds from *B. natalensis* and GC-MS profiling supported by ^{1}H NMR fingerprints provide comprehensive detail of the plant's bioactive metabolites in addition to the isolated compounds. The antioxidant potential of *B. natalensis* may be partly responsible for its use in the management of some oxidative-stress induced diseases.

Key words: Chromatography, free-radicals, knipholone, phytocompounds, spectroscopy

Key messages:
- Isolation and characterization of phytocompounds that have not been previously reported in *Bulbine natalensis*.
- Whole extract characterization using GC-MS and ^{1}H NMR fingerprinting.
- The methanol extractof *B. natalensis* exhibited stronger ferric reducing antioxidant power and, on the other hand, dichloromethane extract of the plant showed stronger Mo(IV) reducing power which was comparable *α*-tocopherol and butylated hydroxytoluene.

INTRODUCTION

Medicinal plants are among nature's most valuable and readily available resources, providing man with preventive and curative regimens for countless diseases all over the world.[1,2] South Africa ranks high among the world's richest plant diversity.[3] Most of these plants are used by traditional medicinal practitioners for managing

different ailments in this country. A comprehensive knowledge of the phytoconstituents of medicinal plants will provide scientific credence to their ethnopharmacological usage and also feed pharmaceutical sectors with varieties of biologically active compounds to produce drugs that meet global health needs.

B. natalensis (Baker) is a medicinal herb which belongs to the family, Asphodelaceae and sub-family, Asphodeloideae.[4] It is a South Africa's indigenous succulent species with wide distribution, not only across Eastern and Northern South Africa but also Swaziland, Mozambique, and Zimbabwe.[4,5] It is a corm-bearing plant with an aloe appearance, but without thorns.[4,5] *B. natalensis* is known traditionally as an aphrodisiac.[6] The leaves are used for treating burns, rashes, ringworms, and wounds, while roots are used to manage convulsion, diabetes, diarrhea, rheumatism, and venereal diseases.[6] Some anthraquinones[7] and tetracyclic triterpenes[8] have been found in *B. natalensis*. However, there is no report of pentacyclic triterpenes from this species. Pentacyclic triterpenes represent a class of phytocompounds whose biological activities are recently gaining more attention as they are being used in complementary medicine and as nutraceuticals.[9] The multifaceted therapeutic potential of plant extracts resulting from their "chemically-complex" but "intelligently-mixed" matrices, make these multi-agent medicines important in modern drug discovery.[1] *B. natalensis* is a plant that has compounds that can target a range of important pharmacological activities.[6] Although extracts from *B. natalensis* have tested positive for alkaloids, anthraquinones, saponins, and tannins,[10] the knowledge of exact phytocompounds in the plant are needed to fully explore its therapeutic potential.

Antioxidants provide protection against diseases such as arthritis, asthma, and diabetes.[11,12] They are substances, mostly of natural origin (but can also be synthetic), with the capacity to either prevent production or scavenge reactive oxygen species, thereby inhibiting oxidative cellular damage.[11] Reactive oxygen species such as hydroxyl radical (OH^-), superoxide anions (O_2^-), and hydrogen peroxide are generated during normal biochemical reactions. The role played by antioxidants in modern drug discovery cannot be overemphasized as antioxidant-based pharmaceutical formulations are recently being developed for preventing and treating diseases including Alzheimer's, cancer, diabetes, neurodegenerative diseases, Parkinson's, and rheumatoid arthritis.[12]

To date, there are only few scientific reports[7,8] on the chemical composition and antioxidant potential of *B. natalensis*. Having identified *B. natalensis* as a medicinal plant species used in the management of some oxidative stress-induced diseases, we aim herein to isolate and characterize biologically active phytocompounds from the plant and evaluate their antioxidant potential alongside the plant's crude extracts.

MATERIALS AND METHODS

General Experimental Procedures

Infrared (IR) spectra were obtained on a PerkinElmer Spectrum 100 Fourier transform IR spectrophotometer with a universal attenuated total reflectance sampling accessory (PerkinElmer, Waltham, USA). ^1H, ^{13}C, and 2D nuclear magnetic resonance (NMR) spectra were recorded using deuterated chloroform ($CDCl_3$) or methanol (CD_3OD) at room temperature on a Bruker Avance III 400 spectrometer (Bruker, Rheinstetten, Germany). Column chromatography was carried out using Merck silica gel 60 (0.040–0.063 mm) and preparatory thin-layer chromatography (PTLC) was carried out using Merck pre-coated Kieselgel 60 (20 cm × 20 cm, 0.5 mm thick) F-254 preparative layer plates while Merck 20 cm × 20 cm silica gel 60 F_{254} aluminum sheets were used for thin-layer chromatography (TLC). The TLC plates were analyzed using an ultraviolet (UV) (254 and 366 nm) lamp before further visualization by spraying with 10% sulfuric acid in methanol solution followed by heating. Alpha-tocopherol standard (≥95.5%) was purchased from Sigma-Aldrich (St. Louis, USA). Solvents (analytical grade) and other chemicals used were supplied by either Merck (Darmstadt, Germany) or Sigma (St. Louis, USA) chemical companies.

Plant Material

The leaves of *B. natalensis* were collected in June 2017 from the Westville Campus of the University of KwaZulu-Natal (UKZN). The plant was identified and authenticated (voucher specimen No: Bodede 03) at the School of Life Sciences, UKZN, Westville, Durban, South Africa.

Extraction, Isolation, and Purification

Leaves of *B. natalensis* were air-dried and extracted sequentially with n-hexane, dichloromethane, and methanol. The resulting crude extracts were subjected to TLC profiling. Hexane and dichloromethane extracts were combined based on the similarity of retention factors (R_f) of phytocompounds. The resulting extract from the combination is referred to as dichloromethane extract in this work. The dichloromethane extract (4 g) was subjected to column chromatography with hexane: ethyl acetate as mobile phase, starting with 100% hexane. The polarity of the mobile phase was gradually increased by 10% ethyl acetate for every 200 mL until 100% ethyl acetate was reached. A total of 30 aliquots was collected and combined into four fractions, namely, A (1–7), B (8–13), C (14–20), and D (20–30). Fraction B afforded compounds **1** (200 mg) (on crystallization from ethyl acetate), **2** (110 mg), and **3** (40 mg) by direct precipitation. Compounds **4** (105 mg) and **5** (80 mg) were obtained through PTLC of fractions C and D, respectively. The mobile phase employed for the PTLC in both fractions was hexane: ethyl acetate (1:1, v/v).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was carried out using a Shimadzu GCMS-QP 2010SE instrument. The instrument was fitted with Shimadzu HP-5MS capillary column (0.25 μm film thickness) having a dimension of 30 m (length) ×0.25 μ (I.D.). Hexane and methanol extracts of *B. natalensis* alongside the standard, *α*-tocopherol, were subjected to GC-MS analysis. Helium was used as the carrier gas at 7.5 kPa pressure with oven temperature programmed at 60°C (for 2 min)–310°C (for 30 min) at a ramping rate of 4°C/min. A 3 μL sample was manually injected (splitless injection) at an injection temperature of 310°C and sample ionization energy of 70 eV was used for GC-MS detection. The system software was driven by Shimadzu GCMS solution workstation software (Japan). The relative amount of each compound in the extracts as a percentage was then computed by comparing the area under the peak for a compound to the total area. Moreover, the chromatogram of *α*-tocopherol was compared with those of the extracts for the evaluation of *α*-tocopherol in the extracts. Having identified the chemical compositions in the extracts, chromatograms were further imported into Mnova software Version 9.0.1.13254 (Mestrelab Research S.L. [USA]) for phase (automatic) and baseline (full auto-Whittaker smoother) corrections. Mnova was also used for the chromatograms stacking and selected area expansion.

Antioxidant Activity

The total reducing power of the compounds from plant material was determined according to the ferric reducing antioxidant power (FRAP) method, as described by Murthy *et al.*[13] with some modifications. A 2.5 mL volume of different concentrations of the plant extracts or compounds were mixed with 2.5 mL phosphate-buffered solution (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$ in test tubes. The mixture was placed in a water bath at 50°C, for 20 min. A volume of 2.5 mL of 10% trichloroacetic acid was added to the mixture and mixed thoroughly. A volume of 2.5 mL of this mixture was then mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%) solution and allowed to stand for 10 min. The absorbance of the mixture was measured at 700 nm using a UV spectrophotometer (Biochrom Libra S11, Cambridge, England); the higher the absorbance of the reaction mixture, the greater the reducing power. Ascorbic acid, butylated hydroxytoluene (BHT), and *α*-tocopherol were used as positive controls. All procedures were performed in triplicate.

A second antioxidant assay, the phosphomolybdenum assay was carried out following the method described by Aliyu *et al.*[14] with some modifications. A 0.3 mL of extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at

95°C for 90 min. Thereafter, the absorbance of the solution was measured at 695 nm using a UV spectrophotometer after cooling at room temperature. Ascorbic acid, BHT, and *α*-tocopherol were used as positive controls. All procedures were performed in triplicate.

Statistical Analysis

All experiments were done in triplicate and expressed as mean ± standard deviation. Separation of means was done by Tukey's *Post hoc* range test obtained using the Statistical Package for the Social Sciences (PASW Statistics, Version 25, IBM Corporation, Cornell, New York). The differences between the means were considered significant for values of $P < 0.05$.

Characterization of Isolated Compounds

Glutinol (**1**): IR (KBr) v_{max} cm^{-1}: 3450, 2917, 2849, 1472, 1462, 1036, 730, 719. ^{1}H NMR (CDCl$_3$, 400 MHz) δ_H: 5.61 (1H, d, $J = 6.0$ Hz, H-6), 3.44 (1H, br s, H-3), 1.14 (3H, s, H-28), 1.11 (3H, s, H-23), 1.07 (3H, s, H-26), 1.02 (3H, s, H-24), 0.98 (3H, s, H-27), 0.96 (3H, s, H-30), 0.93 (3H, s, H-29), 0.82 (3H, s, H-25). ^{13}C NMR (CDCl$_3$, 400 MHz) δ_C: 141.6 (C-5), 122.0 (C-6), 76.3 (C-3), 49.6 (C-10), 47.4 (C-18), 43.0 (C-8), 40.8 (C-14), 39.3 (C-4), 38.9 (C-22), 37.8 (C-13), 36.0 (C-16), 35.0 (C-19), 34.8 (C-9), 34.6 (C-11), 34.5 (C-30), 33.1 (C-21), 32.3 (C-28), 32.08 (C-15), 32.03 (C-29), 30.3 (C-12), 30.0 (C-17), 28.9 (C-23), 28.2 (C-20), 27.8 (C-7), 25.4 (C-4), 23.6 (C-1), 19.6 (C-27), 18.4 (C-26), 18.2 (C-2), 16.2 (C-25).

Taraxerol (**2**): IR (KBr) v_{max} cm^{-1}: 3491, 2916, 2850, 1461, 1416, 1385, 1035, 690. ^{1}H NMR (CDCl$_3$, 400 MHz) δ_H: 5.51 (1H, dd, $J = 8.02, 3.06$ Hz, H-15), 3.18 (1H, dd, $J = 10.97, 4.52$ Hz, H-3), 1.99 (1H, dt, $J = 12.66, 3.04$ Hz), 1.90 (1H, dd, $J = 14.74, 2.89$ Hz) 1.06 (3H, s, H-27), 0.95 (3H, s, H-23), 0.92 (3H, s, H-29), 0.90 (3H, s, H-25), 0.88 (6H, s, H-28 and 30), 0.80 (3H, s, H-26), 0.78 (3H, s, H-24).^{13}C NMR (CDCl$_3$, 400 MHz) δ_C: 158.0 (C-14), 116.8 (C-15), 79.0 (C-3), 55.5 (C-5), 49.2 (C-18), 48.7 (C-9), 41.3 (C-19), 38.9 (C-4), 38.7 (C-8), 37.9 (C-17), 37.7 (C-1), 37.5 (C-13), 36.6 (C-16), 35.7 (C-10), 35.1 (C-12), 33.6 (C-21), 33.3 (C-29), 33.0 (C-22), 29.9 (C-28), 29.8 (C-26), 28.8 (C-20), 27.9 (C-23), 27.1 (C-2), 25.9 (C-27), 21.3 (C-30), 18.7 (C-6), 17.5 (C-11), 15.4 (C-24 and 25).

β-sitosterol (**3**): IR (KBr) v_{max} cm^{-1}: 3406, 2933, 2865, 1463, 1375, 1053, 801, 590. ^{1}H NMR (CDCl$_3$, 400 MHz) δ_H: 5.33 (1H, bd, H-6), 3.51 (1H, m, H-3), 0.98 (3H, s, H-29), 0.90 (3H, d, $J = 6.43$ Hz, H-19) 0.77-0.84 (9H, H-24, 26 and 27), 0.65 (3H, s, H-28), ^{13}C NMR (CDCl$_3$, 400 MHz) δ_C: 140.7 (C-5), 121.7 (C-6), 71.8 (C-3), 56.7 (C-14), 56.0 (C-17), 50.1 (C-9) 45.8 (C-22), 42.3 (C-4 and 13), 39.7 (C-12), 37.2 (C-1), 36.5 (C-10), 36.1 (C-18), 33.9 (C-20), 31.9 (C-7), 31.6 (C-8), 29.7 (C-2) 29.1 (C-25), 28.2 (C-16), 26.0 (C-15), 24.3

(C-21), 23.0 (C-23), 21.0 (C-11), 19.8 (C-26), 19.4 (C-27), 19.0 (C-19), 18.7 (C-28), 11.9 (C-24), 11.8 (C-29).

Knipholone (**4**): IR (KBr) v_{max} cm^{-1}: 3360, 2919, 2850, 1674, 1595, 1454, 1360, 1270, 1205, 1109, 907, 740, 536. ^{1}H NMR (CDCl$_3$, 400 MHz) δ_H: 14.15 (1H, s, OH), 12.57 (1H, s, OH), 11.93 (1H, s, OH), 7.59-7.54 (2H, overlapping dd, H-5 and 6), 7.27 (1H, s, H-2), 7.21 (1H, q, J = 7.45, 1.98 Hz), 6.12 (1H, s, H-5'), 3.93 (3H, s, ArOCH$_3$), 2.63, (3H, s, ArCOCH$_3$), 2.13 (3H, s, ArCH$_3$) δ_C: 203.5 (ArCOCH$_3$), 192.7 (C-9), 182.9 (C-10), 163.2 (C-2'), 163.1 (C-4'), 162.8 (C-6'), 161.8 (C-1), 159.3 (C-8), 152.4 (C-3), 137.1 (C-6), 134.3 (C-5a), 132.8 (C-4a), 125.8 (C-4), 123.9 (C-2), 120.0 (C-7), 115.4 (C-8a), 115.3 (C-1a), 106.1 (C-3'), 104.7 (C-1'), 90.4 (C-5'), 55.5 (OCH$_3$), 33.1 (COCH$_3$), 20.9 (ArCH$_3$).

Pheophytin a (**5**): IR (KBr) v_{max} cm^{-1}: 3388, 2922, 2866, 1734, 1694, 1453, 1364, 1156, 1032, 983, 670. ^{1}H NMR (CDCl$_3$, 400 MHz) δ_H: 9.47 (1H, s), 9.33 (1H, s), 8.53 (1H, s), 7.98 (1H, dd, J = 17.9, 11.5 Hz), 6.28 (2H, dd, J = 17.9, 1.4 Hz), 6.24 (1H, s), 6.16 (2H, dd, J = 11.5, 1.5 Hz), 4.49 (1H, m), 4.19 (1H, d, J = 8.16 Hz), 3.86 (3H, s), 3.37 (3H, s), 3.18 (3H, s), 2.62 (1H, m), 2.47 (1H, m), 2.33 (1H, m), 2.18 (1H, m), 1.79 (3H, d, J = 7.16 Hz), 1.66 (3H, t).

RESULTS AND DISCUSSION

Identification of Isolated Phytocompounds from *B. natalensis*

Structures of isolated compounds are shown in Figure 1 while the ^{1}H, ^{13}C, distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond correlation NMR spectra of the compounds are provided in the Supplementary materials (Figure S1-S18).

Compound **1** was identified as glutinol, a white fluffy solid. Eight singlet methyl groups were observed in its ^{1}H NMR spectrum. Olefinic protons were observed at δ_H 5.61 (C-6) and hydroxylated proton was observed at δ_H 3.44 (C-3). ^{13}C and DEPT spectra showed eight methyl, ten methylene, and seven quaternary carbons. The structure of glutinol was confirmed based on the similarity of chemical shifts with those previously reported from *Tibouchina urvilleana* and *Eugenia brevistyla*.[15,16]

Compound **2** (taraxerol) shared physical and structural similarities to glutinol, as well as being an all-six-member pentacyclic triterpenoid with the same molecular formula
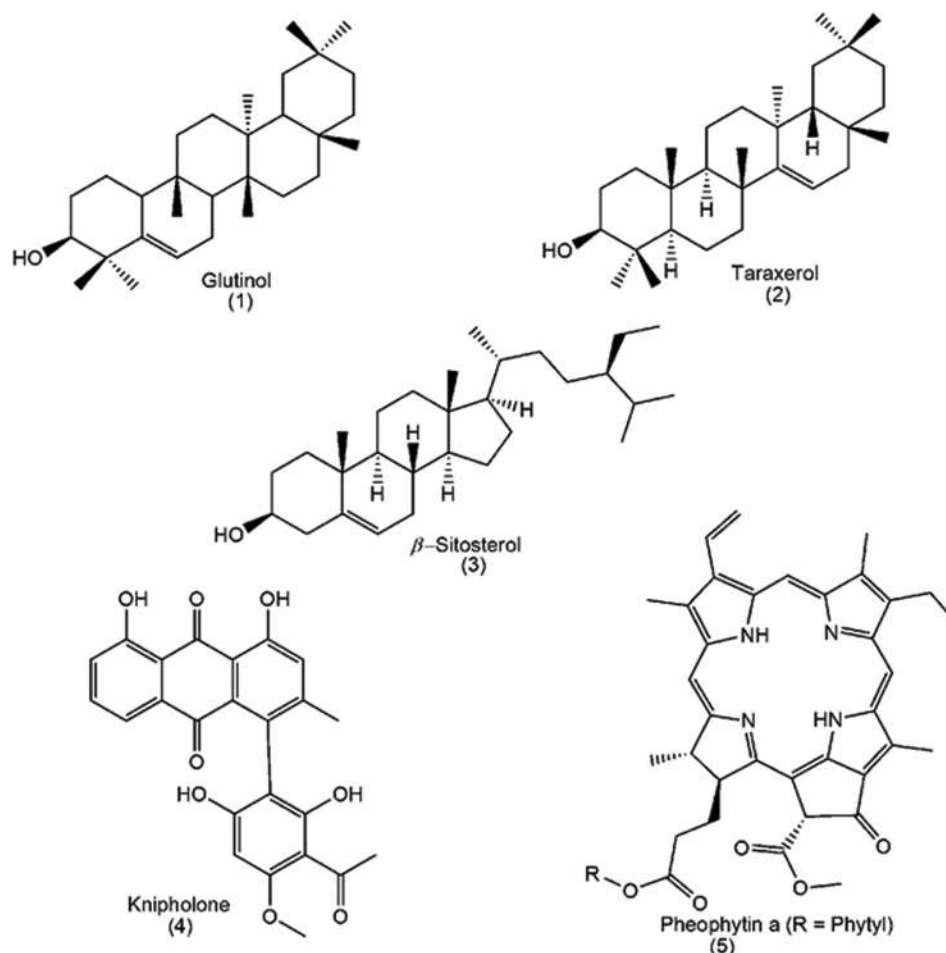

Figure 1: Chemical structures of compounds isolated from *Bulbine natalensis*

and number of carbons on both positive and negative axes of the DEPT spectra. The major structural differences between taraxerol and glutinol are the rearrangement of the methyl groups and the shift in the position of the double bond from position 5 (in glutinol) to 14 (in taraxerol). Thus, the proton at position 3 in taraxerol appeared to be less deshielded (δ_H 3.18) compared to that of glutinol (δ_H 3.44) due to wide separation of the double bond from the proximity of the H-3. The chemical shifts assignments for taraxerol are consistent with those reported by Pérez-Castorena.[15]

Compound **3** (*β*-sitosterol), a ubiquitous phytocompound was obtained as a white amorphous solid. All ¹H and ¹³C NMR chemical shifts were consistent with those previously reported by Chaturvedula and Prakash.[17]

Compound **4** (knipholone) was obtained as a red crystalline solid. The ¹H NMR spectrum showed four protons between δ_H 7.59 and 7.21, indicating a mono-substituted chrysophanol unit while the singlet at δ_H 6.12 (H-5') indicates the monosubstituted (methoxy) acetylphloroglucinol unit. The 20 peaks observed in the ¹³C NMR further justify compound **4** as a phenyl anthraquinone. Both ¹H and ¹³C NMR spectra of knipholone have previously been reported from the species of *Kniphofia*, *Bulbine,* and *Bulbinella*.[18-20]

Compound **5** (pheophytin a) was obtained as a dark green amorphous solid. Its complex NMR spectra were elucidated by comparing the spectra with those reported by Wang *et al*.[21] The highly deshielded methine singlets observed between δ_H 8.5 and 9.4 were characteristic of a penta-substituted porphyrin macrocyclic system. A single, highly shielded N-H signal was also observed at −1.65 ppm due to the fast N-H exchange between the possible tautomeric forms of the porphyrin.[22]

To the best of our knowledge, this is the first report of pentacyclic triterpenoids (glutinol and taraxerol) from *B. natalensis*. Ergosterol, *β*-sitosterol, and stigmasterol are tetracyclic triterpenoids previously isolated from *B. natalensis*.[8] The presence of pentacyclic and tetracyclic triterpenes in *B. natalensis* suggests that the plant is a potential source of these groups of bioactive compounds.

GC-MS Analysis of *B. natalensis* Leaves Crude Extracts

Table 1 shows the profile of additional metabolites of medicinal importance that was obtained from the GC-MS analysis of the dichloromethane and methanol extracts of *B. natalensis*. Figure 2 shows the chromatograms of the dichloromethane and methanol extracts and pure *α*-tocopherol.

The GC-MS analysis of *B. natalensis* leaf extracts revealed more of its chemical composition, among which is *p*-hydroxyphenyacetic acid. This phenolic compound found

in the methanol extract, with the highest percentage (6.70%), has been employed for pharmaceutical preparations.[23] Vitamin E isoforms like the tocopherols are well-known antioxidants.[24] Alpha-tocopherol occurred in both extracts of *B. natalensis* while the beta isomer was only found in the dichloromethane extract. Alpha- and *β*-tocopherol identified in leaf extracts in this study may contribute to the antioxidant activity of *B. natalensis*. A series of long-chain alcohols were identified among which is the plant growth stimulant, triacontanol.[25] This may be responsible for the abundance and high survival rate of *B. natalensis* in Southern Africa.[4,5]

Beta-sitosterol, isolated in its pure form in this study, had the highest percentage composition in the dichloromethane extract (11.27%) and an appreciably high percentage in the methanol extract (6.63%), compared to other identified compounds. Steroidal triterpenes are known to be biosynthetic precursors to androgenic steroids due to the structural similarities of the former to cholesterol.[26,27] In a previous study on the anabolic and androgenic activities of *B. natalensis*, higher levels of testosterone were observed in experimental rats that were treated with aqueous extracts of the plant.[6] This may be due to the presence of steroidal triterpenes such as *β*-sitosterol, campesterol, and the previously isolated ergosterol and stigmasterol. Some of the other chemical composition observed [Table 1] are biosynthetic precursors, intermediates or degradation products of several important secondary metabolites[28,29] which were not isolated in this study. However, the synergistic effects of these compounds may be responsible for the bioactivity of *B. natalensis* in its traditional medicinal usage.

¹H NMR Fingerprints of *B. natalensis* Leaves Crude Extracts

The metabolites identified using GC-MS were further verified using chemical shift annotations on the ¹H NMR spectra of the dichloromethane and methanol extracts of *B. natalensis* leaves [Figure 3]. The fingerprints showed chemical shifts ranging from 0.50 to 9.75 ppm. Compounds annotation was possible using chemical shifts reported in the literature and standard online databases,[17,30-46] as revealed in Table 1. The structures of selected annotated biologically-active compounds are presented in Figure 4. The selection was based on prominent peak areas and compounds of special biological interest.

Antioxidant Activity of Isolated Compounds and Crude Extracts from *B. natalensis*

The FRAP of the standards, extracts, and compounds of the leaves of *B. natalensis* is shown in Figure 5. Ascorbic acid had the strongest antioxidant activity overall; it was removed from the figure so as to better represent the extracts and compounds against the other standards (BHT and *α*-tocopherol). All compounds and crude extracts showed

concentration-dependent reducing power. This observation is similar to previous work carried out on *Bulbine frutescens*.[47]
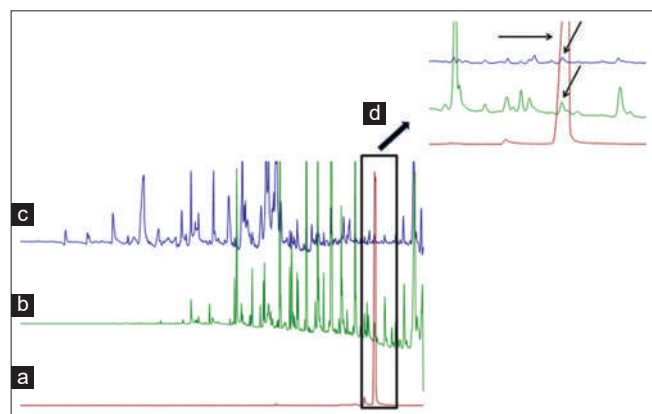

Figure 2: Gas chromatography-mass spectrometry chromatograms of α-tocopherol (a), dichloromethane extract of *Bulbine natalensis* leaves (b), methanol extract of *B. natalensis* leaves (c), and expanded α-tocopherol region (d)

The reducing power (at 250 µg/mL) was in the decreasing order of ascorbic acid > BHT > methanol extract > α-tocopherol > pheophytin a > glutinol = β-sitosterol = dichloromethane extract > taraxerol > knipholone. FRAP assay employs the ability of the test compound or extracts to reduce Fe^{3+} to Fe^{2+}. It is likely that most of the participating compounds in the methanol extract (with the highest ferric reducing power compared to other samples) possess antioxidant properties with electron-donating ability. This is indicative of the presence of phenolics and other polar components in the methanol (polar solvent) extract. Research has shown antioxidant activity to be enhanced when the hydroxyl group at C-3 is in close proximity to the double bond at C-5 and C-6 as in sterols and triterpenes.[48] This could explain why glutinol had higher antioxidant activity than taraxerol in this study.

Figure 6 shows the concentration versus absorbance curves of standards, extracts, and compounds, as obtained by the phosphomolybdenum assay. Ascorbic acid had the strongest

Table 1: Chemical composition of *Bulbine natalensis* leaf extracts obtained from GC-MS analysis and ^{1}H NMR

Chemical composition	Rt*(min)	MW	Extracts (% peak area)		^{1}H NMR profile	
			DCM extract	MeOH extract	Chemical shift (ppm)	Reference
Tyrosol	11.54	138	-	1.94	7.09, 6.77, 3.83, 2.8 2	[30]
p-Hydroxyphenylacetic acid	13.37	152	-	6.70	7.12, 6.78, 3.51	[31]
Nonanedioic acid	14.25	188	-	1.01	2.30, 1.52, 1.29	[32]
Pentadecanal	16.18	226	0.41	2.10	9.79, 2.43, 1.36, 0.90	[33]
Citronellyl propionate	16.63	212	-	0.40	5.20, 4.08, 2.29, 1.96, 1.82, 1.70, 1.65, 1.54, 1.53, 1.14, 0.96	[34]
Palmitic acid	17.52	256	-	2.39	2.20, 1.60, 1.20, 0.85	[35]
Trans-Phytol	18.90	296	2.99	0.41	5.41, 4.15, 2.00, 1.67, 1.25-1.62, 0.85–0.87	[36]
cis-Oleic acid	19.23	282	-	5.28	5.34, 2.33, 2.01, 1.63, 1.28, 0.88	[37]
Stearic acid	19.41	284	-	1.51	2.35, 1.64, 1.26, 0.88	[38]
Stearaldehyde	20.78	268	0.46	-	9.72, 2.40, 1.58, 1.31, 1.29, 1.26, 0.88	[39]
Stearyl acetate	21.48	312	8.83	-	4.05, 2.03, 1.60, 1.26, 0.88	[40]
n-Docosanol	22.17	326	2.69	0.82	3.63, 1.57, 1.47, 1.26, 0.89	[41]
α-Monostearin	22.34	358	0.35	-	4.19, 4.13, 3.92, 3.69, 3.59, 2.34, 1.62, 1.28–1.24, 0.87	[42]
Octacosanol	23.72	410	4.75	1.40	3.62, 1.55, 1.23, 0.86	[43]
β-Tocopherol	26.26	416	0.26	-	6.46, 4.35, 2.60, 2.09, 2.08, 1.80, 1.50–1.10, 0.88–0.83	[44]
α-Tocopherol	27.09	430	0.42	0.33	4.18, 2.60, 2.15, 2,11, 1.80, 1.50–1.10, 0.88–0.83	[44]
Campesterol	28.35	400	0.86	0.36	5.34, 3.51, 2.28, 1.01, 0.92, 0.84, 0.81, 0.80, 0.68	[45]
Triacontanol	28.85	438	2.21	1.03	3.64, 1.55, 1.25, 0.90	[46]
β-Sitosterol	29.49	414	11.27	6.63	5.36, 3.53, 1.01, 0.93, 0.84, 0.83, 0.81, 0.68	[17]

*Rt: Retention time, DCM: Dichloromethane, MeOH: Methanol, GC-MS: Gas chromatography-mass spectrometry, NMR: Nuclear magnetic resonance
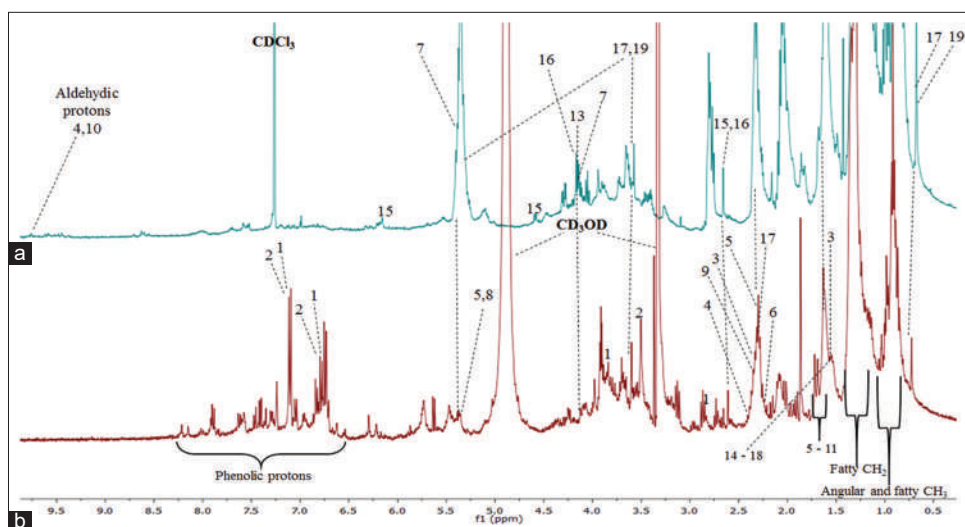
Figure 3: ¹H nuclear magnetic resonance spectra of *Bulbine natalensis* leaf extracts; (a)–dichloromethane extract run in deuterated chloroform, (b)–methanol extract run in deuterated methanol. Tyrosol (1), *p*-Hydroxyphenylacetic (2), nonanedioic acid (3), pentadecanal (4), citronellyl propionate (5), palmitic acid (6), *Trans*-Phytol (7), *cis*-Oleic Acid (8), stearic acid (9), stearaldehyde (10), stearyl acetate (11), n-Docosanol (12), α-Monostearin (13), octacosanol (14), β-Tocopherol (15), α-Tocopherol (16), campesterol (17), Triacontanol (18), β-sitosterol (19)
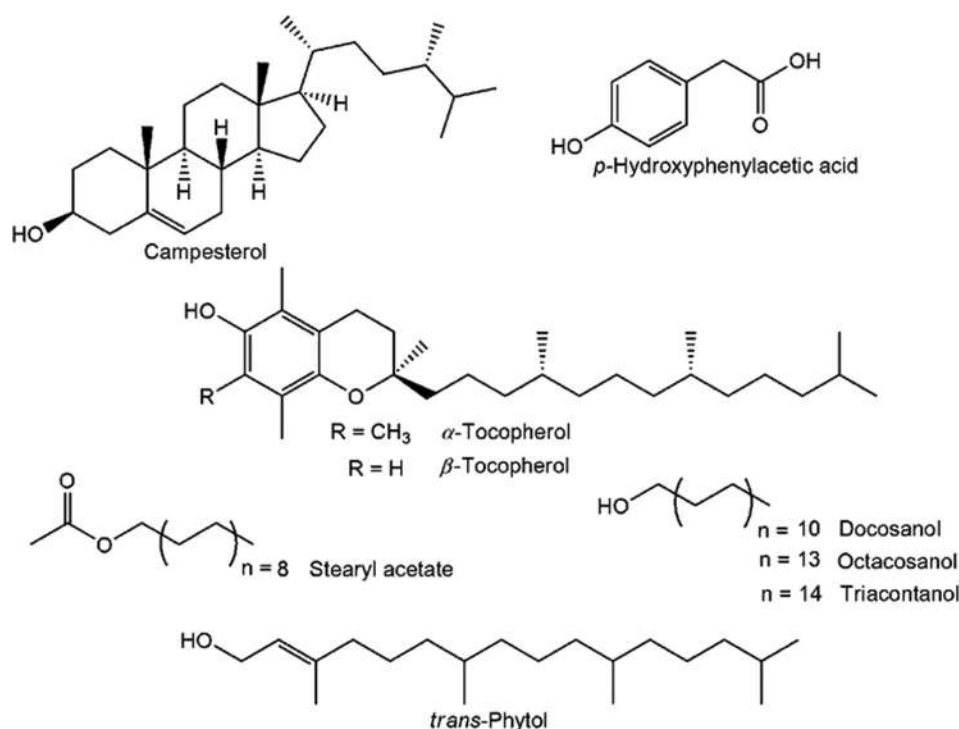
Figure 4: Selected phytocompounds identified in the extracts of *Bulbine natalensis* using gas chromatography-mass spectrometry

antioxidant activity but is not included in the figure as in the case of FRAP. The dichloromethane extract had stronger reducing power compared to the methanol extract. The antioxidant activity (at 250 µg/mL) was in the decreasing order of ascorbic acid > α-tocopherol > pheophytin a > dichloromethane extract > β-sitosterol > glutinol > methanol extract > BHT > knipholone > taraxerol. Tocopherols are good reducing agents of Mo(VI).[49] It is likely that higher percentage of tocopherol in dichloromethane extract is partly responsible for the higher reducing activity compared to the methanol extract. The conjugated pi bonds in the porphyrin ring of pheophytin a, enabled them to act as antioxidants through electron transfer mechanisms.[50] In this study, pheophytin a had an antioxidant activity which was higher than BHT and other isolated compounds but comparable to the known antioxidant, α-tocopherol. This is similar to the previous study where pheophytin showed notable antioxidant activity comparable to a known antioxidant, β-carotenoid.[50]
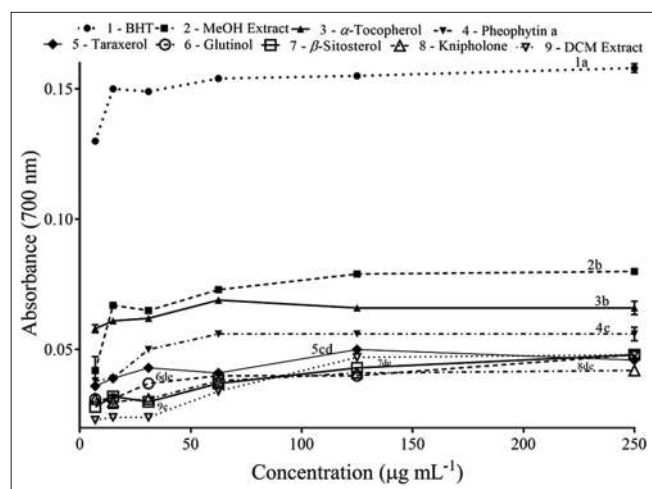
Figure 5: Ferric reducing antioxidant power of extracts and compounds from *Bulbine natalensis*. Means with the same letter(s) are not significantly different by Tukey's *post hoc* test at the 5% level (mean ± standard deviation; $n = 3$)
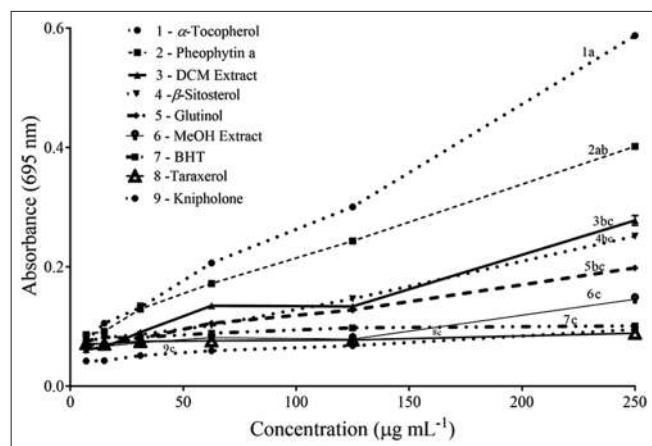

Figure 6: Phosphomolybdenum assay of extracts and compounds from *Bulbine natalensis*. Means with the same letter(s) are not significantly different by Tukey's *post hoc* test at the 5% level (mean ± standard deviation; $n = 3$)

Knipholone, the phenyl anthraquinone, had low antioxidant activity, similar to an earlier report by Wube *et al.*[51] This low antioxidant activity is due to substituents on the benzene ring that lowers the electron density of its hydroxyl groups. Although knipholone has been identified as potential candidate for developing new anti-asthma drugs, Wube *et al.* proposed a non-redox mechanism of action for knipholone due to its failure to scavenge the free radicals of 1,1-diphenyl-2-picrylhydrazyl.[51] Therefore, further studies are required to understand the mechanism of action of knipholone for the development of drugs against oxidative stress-induced diseases.

CONCLUSION

A phytochemical investigation of *B. natalensis* led to the identification of pentacyclic triterpenes (glutinol and taraxerol) which have not previously been reported from the plant. Knipholone, a phenyl anthraquinone, and the biologically-important pigment, pheophytin a, were also isolated. Analysis of extracts by GC-MS and ^{1}H NMR revealed the presence of metabolites whose synergistic effects with isolated compounds are likely to be responsible for the therapeutic potency of *B. natalensis* in African traditional medicine. The methanol extract exhibited stronger FRAP while the dichloromethane extract showed stronger Mo(IV) reducing power which was comparable to the standards, α-tocopherol, and BHT. The study shows *B. natalensis* to be a natural source of antioxidants and this property may be responsible for its multi-faceted medicinal uses.

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Chingwaru W, Kapewangolo P. *In vitro* anti-HIV and antioxidant potential of *Bulbine frutescens* (*Asphodelaceae*). J Herb Med 2017;12:73-78.

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Source of Support: Nil. **Conflicts of Interest:** None declared.

SUPPLEMENTARY MATERIALS
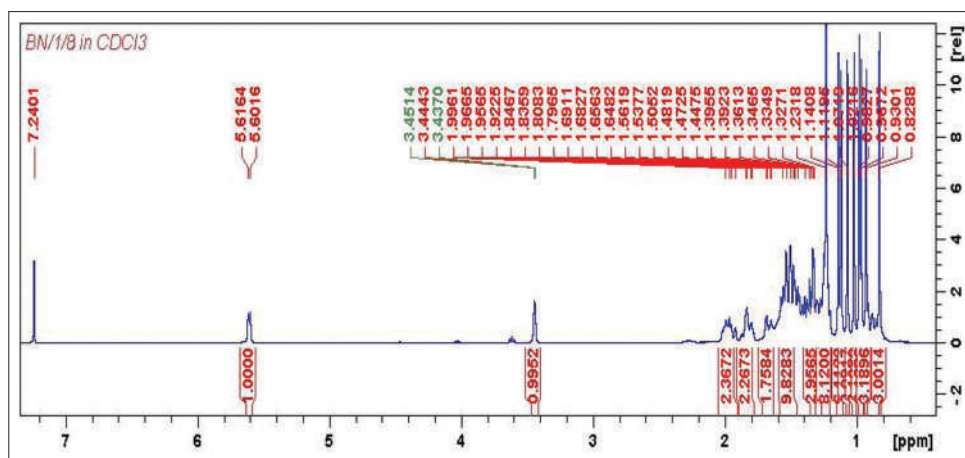
Figure S1: ¹H NMR spectrum of glutinol
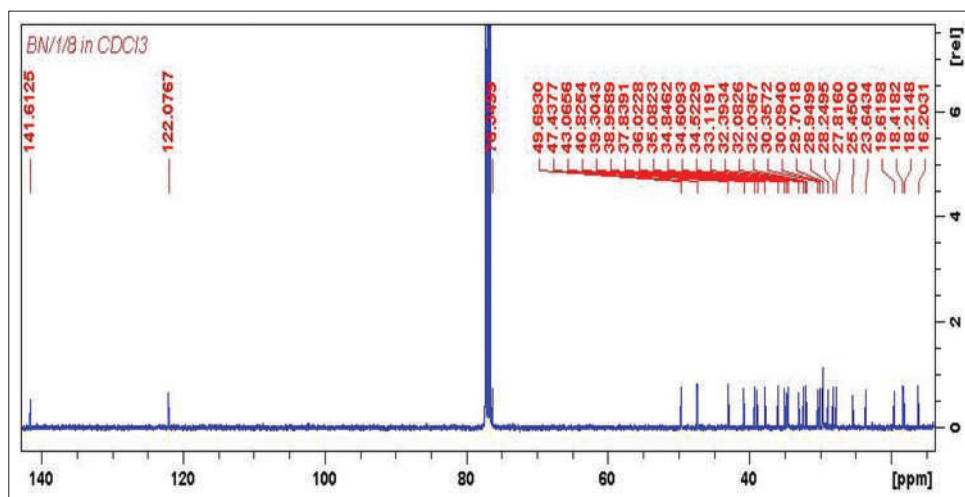
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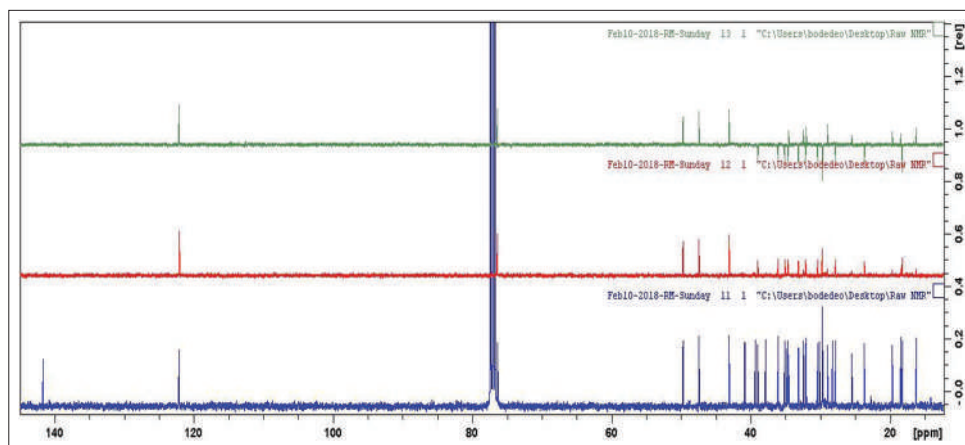
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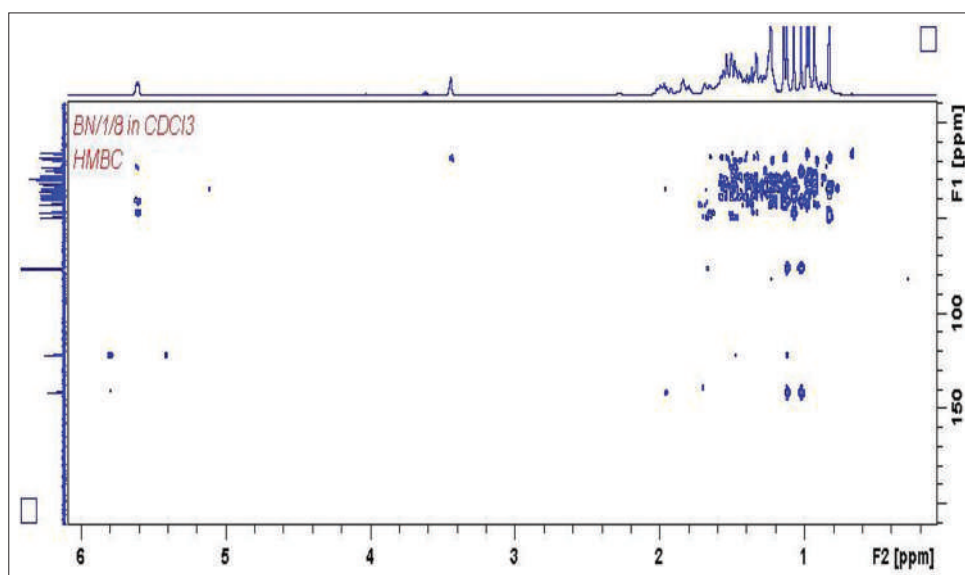
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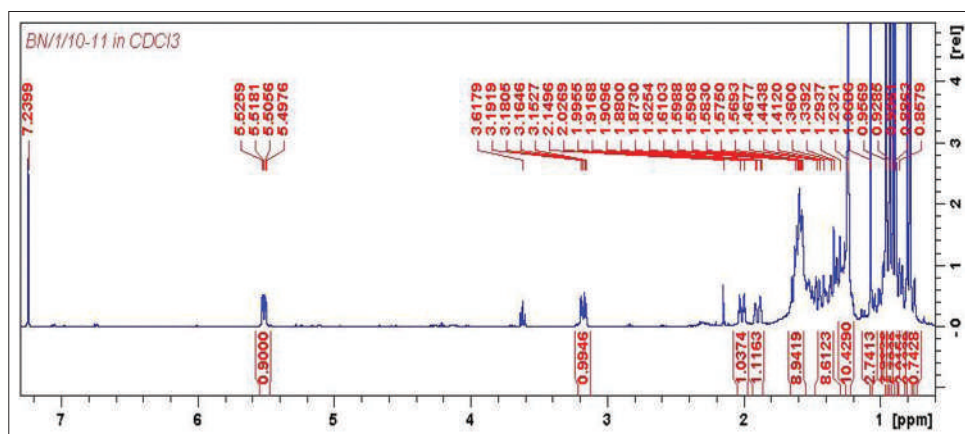
Figure S5: ^{1}H NMR spectrum of taraxerol
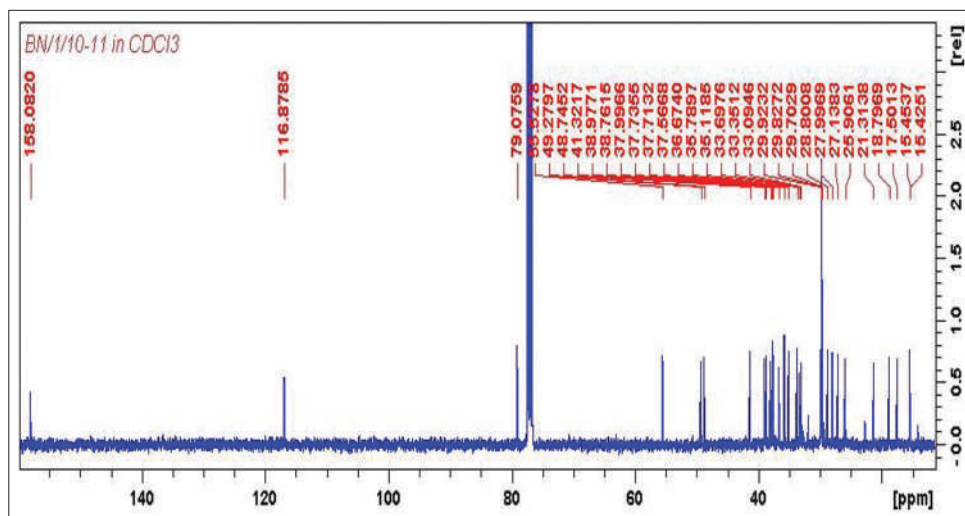
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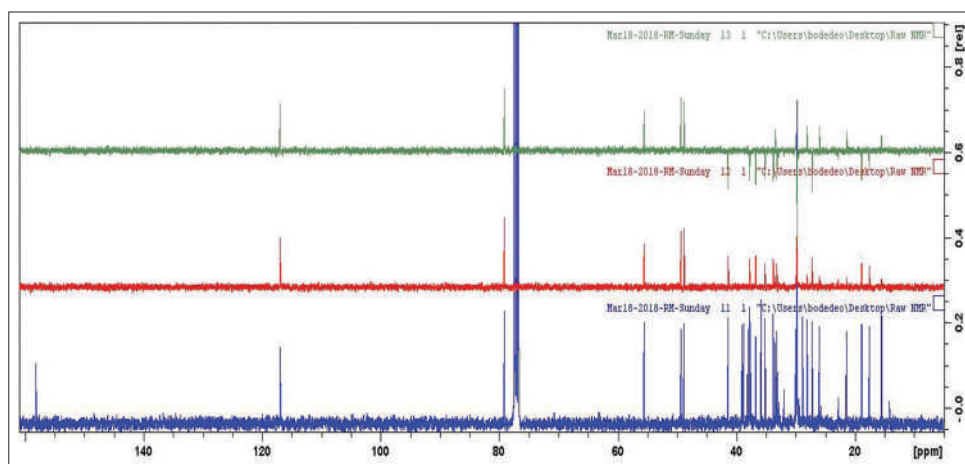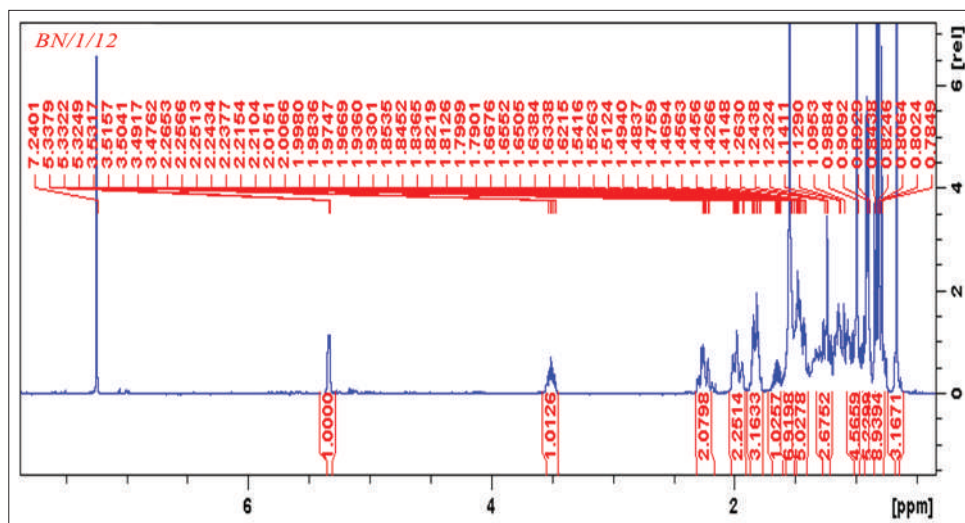

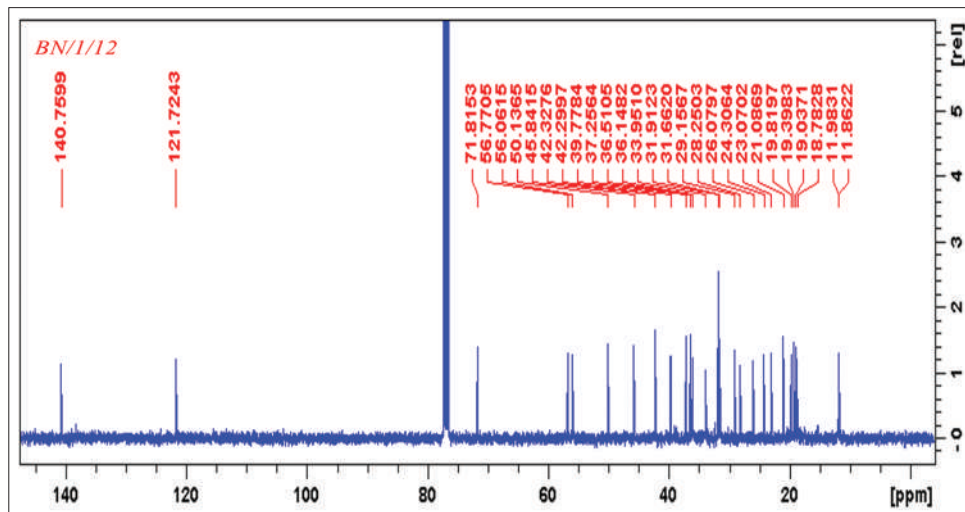**Figure S7:** DEPT spectrum of taraxerol
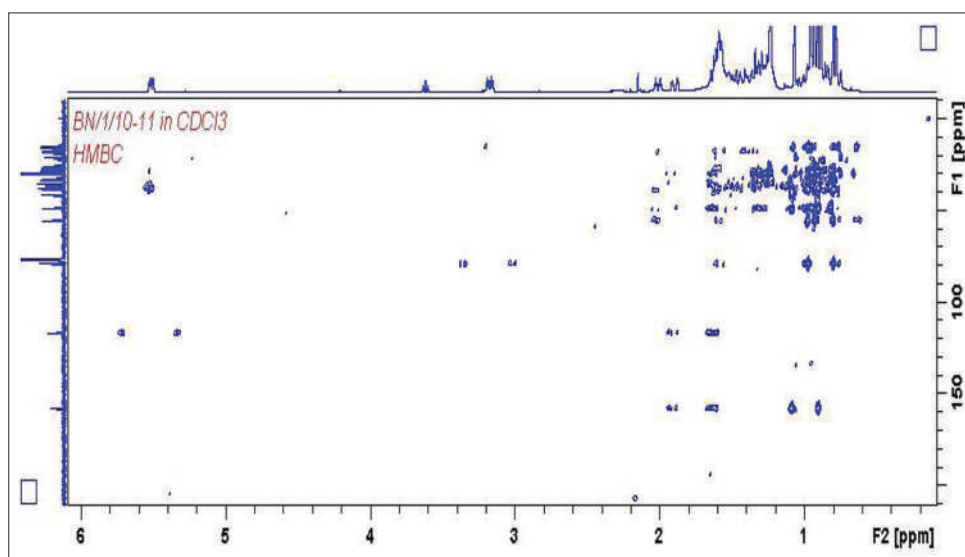
Figure S8: HMBC spectrum of taraxerol

Figure S9: ^{1}H NMR spectrum of β-sitosterol

Figure S10: ^{13}C NMR spectrum of β-sitosterol

Figure S11: ^{1}H NMR spectrum of knipholone
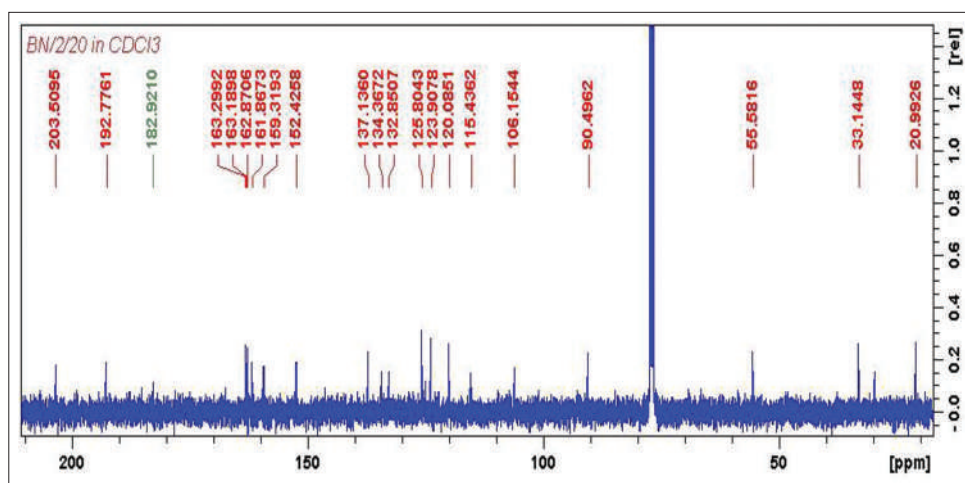
Figure S12: ^{13}C NMR spectrum of knipholone
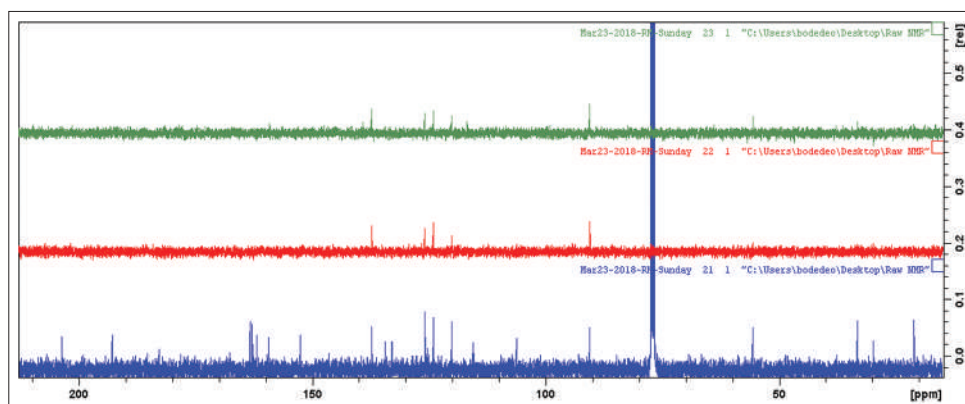
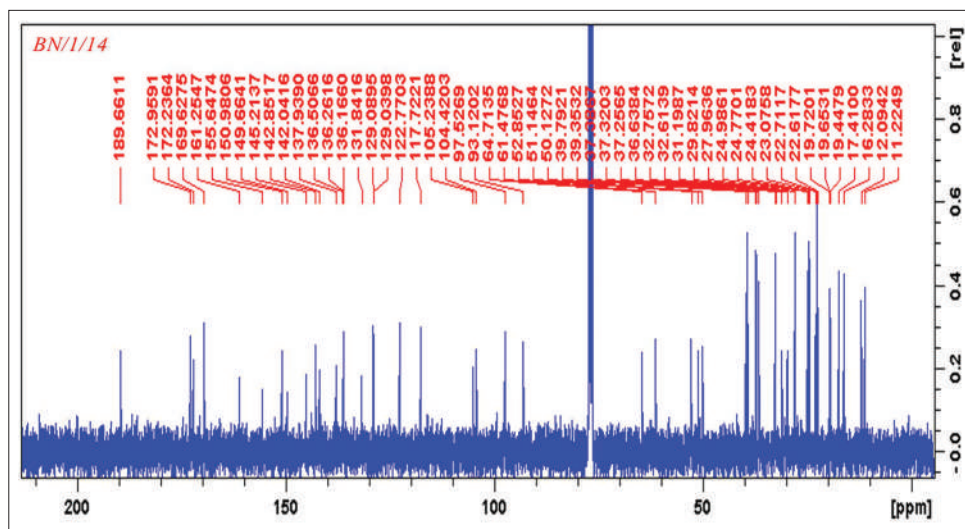**Figure S13:** DEPT spectrum of knipholone
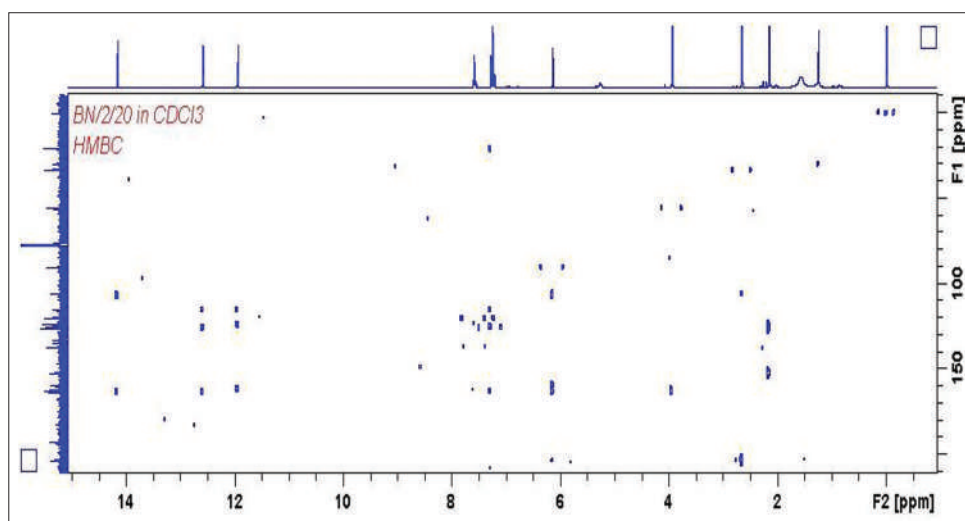
Figure S14: HMBC spectrum of knipholone
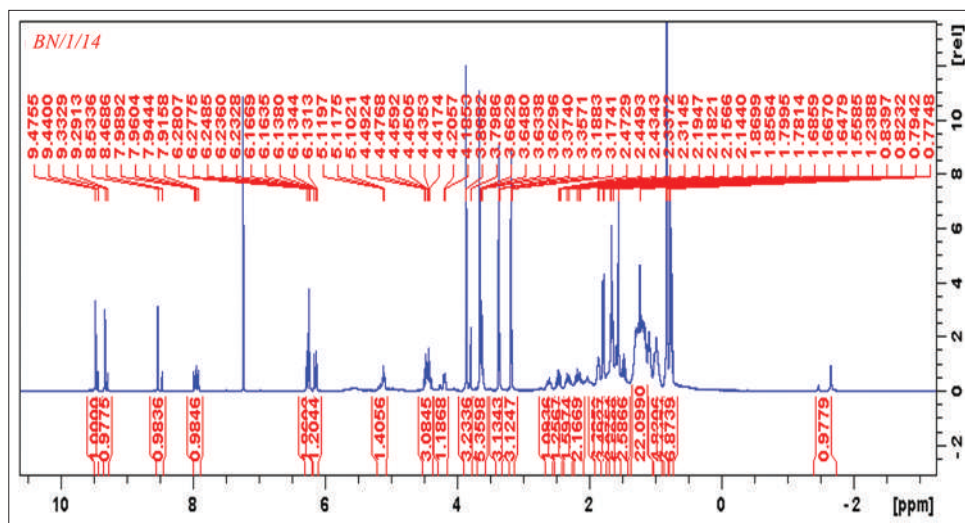
Figure S15: ^{1}H NMR spectrum of pheophytin a

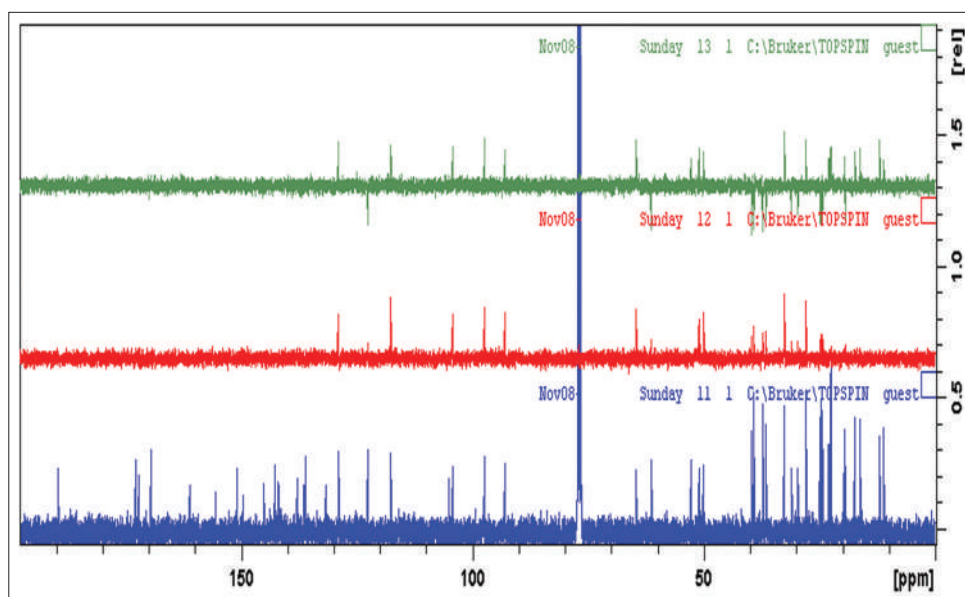**Figure S16:** ^{13}C NMR spectrum of pheophytin a
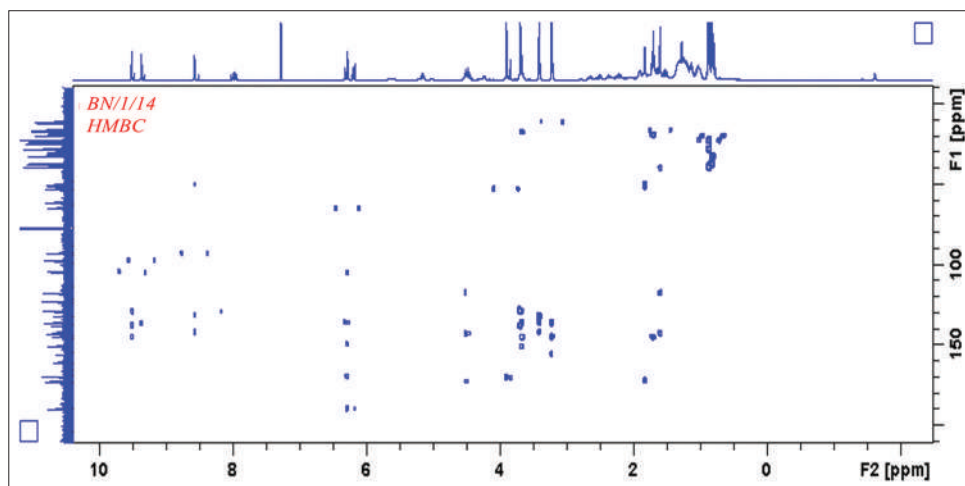
Figure S17: DEPT spectrum of pheophytin a

Figure S18: HMBC spectrum of pheophytin a