Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2018; 10(1); 25-37
doi: 10.25258/phyto.10.1.6
ISSN: 0975-4873

Research Article

# Antibacterial and Anticancer Activity of Ethnomedicinal Plants Used in the Jongilanga Community, Mpumalanga

Lall N[1*], de Canha MN[1], Reid A[1], Oosthuizen CB[1], Langhansova L[1,2], Mahore J[1,3], Winterboer S[1,4], Hamilton C[1,5], Kumar V[1], Gasa N[1], Twilley D[1]

[1]*Department of Plant and Soil Sciences, University of Pretoria, Pretoria, South Africa, 0002*
[2]*Laboratory of Plant Biotechnologies, Institute of Experimental Botany AS CR, v.v.i., Prague 6-Lysolaje, Czech Republic*
[3]*Jongilanga Traditional Council, P. Bag x7401, Ximhungwe 1281, Ehlanzeni District, Mpumalanga, South Africa*
[4]*Guides and Trackers, P.O. Box 3053, The Reeds 0158, South Africa*
[5]*School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, UK, NR4 7TJ*

***Received: 26th Jul, 17; Revised 15th Dec, 17, Accepted: 10th Jan, 18; Available Online:25th Jan, 18***

**ABSTRACT**

Seventy-four ethanolic extracts were prepared from traditionally used medicinal plants in the Jongilanga community in Mpumalanga South Africa. The aim was to determine the biological activity of the selected plants against cancer, mycobacteria species and acne. From the results, it was evident that *Mundulea sericea* was able to inhibit the proliferation of human melanoma cells (A375) with a fifty percent inhibitory concentration ($IC_{50}$) ranging between 50 and 100 µg/ml as well as the ability to inhibit *Mycobacterium tuberculosis*, *Mycobacterium smegmatis* and *Propionibacterium acnes* with minimum inhibitory concentrations (MIC) of 125, 31.25 and 7.9 µg/ml respectively. This further led to the investigation of the antioxidant and anti-inflammatory activity as well as the influence of the extract on mycothiol disulphide reductase (Mtr) and glutathione reductase enzymes (Gtr) as potential targets against the above-mentioned diseases. *M. sericea* inhibited the COX-2 enzyme, responsible for inflammation, with an $IC_{50}$ value of 10.70 ± 1.14 µg/ml, furthermore compounds previously isolated from *M. sericea* showed potential inhibition of COX-2 in molecular docking studies. Low radical scavenging capacity against the DPPH free radical with an $IC_{50}$ value of 60.52 ± 2.40 µg/ml was obtained, however, *M. sericea* showed a higher affinity towards Mtr as compared to Gtr, which makes it an ideal plant for use as an antimycobacterial agent.

**Keywords**: Melanoma; *Mycobacterium tuberculosis*; *Propionibacterium acnes*; cyclooxygenase-2; Molecular docking; Mycothiol disulfide reductase; *Mundulea sericea*.

## INTRODUCTION

Traditional medicine is still one of the primary sources of healthcare in South Africa, with an estimated 27 million individuals relying on traditional medicine. It has been reported that the primary use of traditional medicine is not primarily due to inaccessibility of Western medicine but is due to preference. The trade of traditional medicine results in approximately R2.9 billion, which equates to the usage of an estimated 20,000 tonnes of plant material per year. Most of the plants that are used are indigenous, with a variety of plant parts being used, including bark (27%), roots (27%), bulbs (14%), whole plant (13%), leaves and stems (10%), tubers (6%) and a mixture of parts (3%)[1].

The Cancer Association of South Africa (CANSA) has reported that there is an average lifetime risk of one in every nine females (1:8) and one in every eight males (1:7) to be diagnosed with cancer in South Africa. Furthermore, skin cancer in South Africa accounts for 20,000 new cases and 700 deaths and is the most common cancer in South Africa[2]. Furthermore, a study on dermatological disorders in Johannesburg in 2003 based on 1999 data showed that out of a sample size of 7029 people, acne was the second most prevalent skin disorder making up 16% of the surveyed population. A more recent study in Kwazulu-Natal showed that acne vulgaris makes up 10.3% of the surveyed patients, and other forms of acne account for 44.3% of the recorded diseases[3,4]. Lastly, the WHO in 2013 identified South Africa as one of the countries with the highest rate of TB-infected individuals, with 450,000 new reported cases[5]. Stats SA recorded for the period between 2011-2013, that communicable diseases such as tuberculosis and pneumonia were the leading causes of death across the country with tuberculosis (TB, MDR-TB and XDR-TB) accounting for 8.8% thereof[6].

The selected plant species in this study were therefore, evaluated *in vitro* for activity against *Mycobacterium smegmatis* (non-pathogenic strain), *Mycobacterium tuberculosis* (pathogenic strain) and *Propionibacterium acnes* for anti-TB and anti-acne activity respectively. Anti-cancer activity was investigated against malignant melanoma (A375 cells). Further investigations were performed on *Mundulea sericea* where the highest activity against the above mentioned disorders was observed. Studies included anti-inflammatory activity using

**Author for Correspondence: namrita.lall@up.ac.za*

cyclooxygenase-2 (COX-2) enzyme, antioxidant activity against 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) and inhibitory activity against glutathione reductase and mycothiol disulfide reductase, enzymes responsible for maintaining oxidative stress in humans and *M. tuberculosis* respectively.

In this study, medicinal plants were selected from a database of plants and treatments practiced by traditional healer (TDr) James Mahore that falls under the Jongilanga tribal council, Bushbuckridge municipality, Mpumalanga province, South Africa.

Table 1: Cytotoxicity and antimicrobial activity of selected plant species

| Plant sample | Plant parts | Pru number | *M. smegmatis* | *M. tuberculosis* | *P. acnes* |
|---|---|---|---|---|---|
| | | | MIC[a] in µg/ml | | |
| *Abrus precatorius* L. /Fabaceae | Aerial parts & seeds | BCM119334 | 1000 | 250 | >500 |
| *Acacia karroo* Hayne/ Fabaceae | Roots | BCM119360 | 1000 | 250 | 125 |
| *Acacia nigrescens* Oliv. / Fabaceae | Root bark | BCM117176 | 1000 | NT | 500 |
| *Antidesma venosum* E. Mey. ex. Tul./ Euphorbiaceae | Root bark | BCM117167 | >1000 | NT | 250 |
| *Antidesma venosum* E. Mey. ex. Tul./ Euphorbiaceae | Leaves | BCM117167 | >1000 | NT | >500 |
| *Asparagus buchananii* Baker/ Asparagaceae | Leaves/thorns/stems | BCM119329 | 1000 | 500 | >500 |
| *Asparagus exuvialis* Burch./ Asparagaceae | Stems and roots | BCM119347 | 1000 | 1000 | >500 |
| *Barleria affinis* C.B. Clarke/ Acanthaceae | Roots | BC119 | >1000 | NT | >500 |
| *Blepharis subvolubilis* C.B. Clarke var. *subvolubilis*/ Acanthaceae | Whole plant | BC129 | 250 | 1000 | >500 |
| *Boophone disticha* (L.f.) Herb./ Amaryllidaceae | Leaves/bulb/roots | BC54 | >1000 | >1000 | >500 |
| *Carissa edulis* (Forssk.) Vahl./ Apocynaceae | Roots/stems/leaves | BCM119351 | >1000 | >1000 | >500 |
| *Cassia abbreviata* subsp. beareana (Holmes) Brenan/ Fabaceae | Roots | BC124 | >1000 | >1000 | >500 |
| *Chamaecrista capensis* (Thunb.) E. Mey./ Fabaceae | Roots | BCM119343 | >1000 | >1000 | 62.5 |
| *Clematis brachiata* Thunb./ Ranunculaceae | Roots/leaves/stems | BC73 | >1000 | NT | >500 |
| *Combretum apiculatum* (Sond.) subsp. apiculatum/ Combretaceae | Leaves and stems | BCM119358 | 500 | 250 | 125 |
| *Combretum collinum* Fresen./ Combretaceae | Leaves | BCM117156 | >1000 | >1000 | 500 |
| *Combretum imberbe* Wawra/ Combretaceae | Stems and leaves | BCM117175 | 250 | 125 | 31.25 |
| *Commelina benghalensis* L./ Commelinaceae | Whole plant | BC154 | >1000 | 250 | >500 |
| *Commiphora africana* (A. Rich.) Endl./ Burseraceae | Roots | BC111 | >1000 | NT | >500 |
| *Cordia ovalis* R. Br./ Boraginaceae | Roots | BCM117159 | NT | 1000 | >500 |
| *Crabbea hirsuta* Harv. / Acanthaceae | Roots/leaves/flowers | BCM119366 | >1000 | >1000 | >500 |
| *Crossandra greenstockii* S. Moore/ Acanthaceae | Stems | BC110 | 1000 | 1000 | >500 |
| *Crotalaria agatiflora* Schweinf. / Fabaceae | Roots | BCM119344 | 250 | 125 | >500 |
| *Dalbergia melanoxylon* Guill. & Perr./ Fabaceae | Roots | BCM117154 | >1000 | 500 | 250 |
| *Dicerocaryum eriocarpum* (Decne.) Abels/ Pedaliaceae | Aerial parts | BCM119332 | >1000 | NT | >500 |
| *Dichrostachys cinerea* var. *nyassana* (Taub.) Brenan/ Fabaceae | Pods | BCM117157 | >1000 | NT | 500 |
| *Dichrostachys cinerea* var. *nyassana* (Taub.) Brenan/ Fabaceae | Roots | BCM117157 | >1000 | NT | 125 |

| | | | | | |
|---|---|---|---|---|---|
| *Diospyros lycioides* Desf. var. *lycioides*/ Ebenaceae | Roots | BCM119336 | 500 | 250 | >500 |
| *Diospyros mespiliformis* Hochst. ex. A.D.C/ Ebenaceae | Leaves | BCM117182 | 500 | 1000 | 250 |
| *Drimiopsis burkei* Baker./ Hyacinthaceae | Bulb | BC62 | 1000 | NT | 250 |
| *Gazania krebsiana* Less. var. *krebsiana*/ Asteraceae | Roots/flowers/leaves | BCM119369 | 1000 | 250 | >500 |
| *Gladiolus elliotii* Baker./ Iridaceae | Stems/leaves/bulb | BCM119353 | >1000 | 1000 | <3.91 |
| *Grewia occidentalis* L. / Malvaceae | Leaves | BCM117158 | >1000 | 1000 | >500 |
| *Gymnosporia buxifolia* (L.) Szyszyl/ Celestraceae | Aerial parts, no flowers | BCM117155 | >1000 | >1000 | 500 |
| *Helichrysum pallidum* DC./ Asteraceae | Roots | BCM119348 | >1000 | NT | >500 |
| *Indigofera arrecta* A. Rich./ Fabaceae | Leaves and stems | BCM119331 | 500 | 125 | >500 |
| *Ipomoea crassipes* Hook/ Convolulaceae | Roots | BC162 | >1000 | NT | >500 |
| *Jasminum abyssinicum* Hochst. ex. DC./ Oleaceae | Whole plant | BCM119364 | >1000 | NT | >500 |
| *Jasminum fluminense* Vell. subsp. *fluminense*/ Oleaceae | Roots | BCM119350 | >1000 | NT | >500 |
| *Jatropha zeyheri* Sond./ Euphorbiaceae | Bulb | BC117 | >1000 | NT | >500 |
| *Kalanchoe thyrsiflora* Harv./ Crassulaceae | Leaves/stems/roots | BCM117166 | >1000 | NT | 500-250 |
| *Laggera crispate* (Vahl) Hepper & J.R.I. Wood/ Asteraceae | Roots | BCM119337 | 1000 | >1000 | >500 |
| *Laggera crispate* (Vahl) Hepper & J.R.I. Wood/ Asteraceae | Flowers/leaves/stems | BCM119352 | >1000 | 1000 | 125 |
| *Lannea schweinfurthii* var. *stuhlmannii* (Engl.) Kokwaro/ Anacardiaceae | Root bark | BCM119341 | >1000 | NT | 125 |
| *Ledebouria cooperi* (Hook.f) Jessop./ Hyacinthaceae | Leaves and bulb | BC112 | >1000 | >1000 | >500 |
| *Lippia javanica* (Burm.f.) Spreng/ Verbenaceae | Leaves/stems/seeds | BCM119365 | >1000 | 250 | >500 |
| *Macrotyloma maranguense* (Taub.) Verdc./ Fabaceae | Leaves/stems/roots | BCM117171 | 1000 | 1000 | >500 |
| *Mundulea sericea* (Willd.) A. Chev./ Fabaceae | Roots | BCM119368 | 31.25 | 125 | 7.9 |
| *Ochna natalitia* (Meisn.) Walp./ Ochnaceae | Aerial parts | BCM118701 | >1000 | >1000 | 125 |
| *Opuntia ficus-indica* (L.) Mill./ Cactaceae | Stems and thorns | BCM117178 | 1000 | 250 | >500 |
| *Ormocarpum trichocarpum* (Taub.) Engl./ Leguminosae | Bark | BCM117168 | 500 | NT | 125-62.5 |
| *Ornithogalum seineri* (Engl. & Krause) Oberm./ Hyacinthaceae | Bulb | BC183 | >1000 | >1000 | >500 |
| *Pachypodium saundersii* N.E.Br./Apocynaceae | Leaves/stems/roots | BC116 | >1000 | 500 | >500 |
| *Pappea capensis* Eckl. & Zeyh. / Sapindaceae | Bark | BCM118702 | 500 | 1000 | >500 |
| *Pavetta gracilifolia* Bremek./ Rubiaceae | Roots/stems/leaves | BCM119349 | >1000 | >1000 | >500 |
| *Pavetta gracilifolia* Bremek./ Rubiaceae | Leaves | BCM120573 | NT | NT | >500 |
| *Philenoptera violacea* (Klotzsch) Schrire/ Fabaceae | Roots | BCM119335 | >1000 | 250 | >500 |
| *Phyllanthus reticulatus* Poir. var *reticulatus*/ Euphorbiaceae | Stems/leaves/bark/seeds | BCM118705 | >1000 | >1000 | >500 |
| *Raphionacme procumbens* Schltr./ Asclepiadaceae | Bulb | BCM117172 | >1000 | >1000 | >500 |
| *Rhoicissus tridentata*. var. *cuneifolia* (Eckl. & Zeyh) Urton/ Vitaceae | Roots | BCM119338 | >1000 | NT | 250 |
| *Senna italica* Mill. var. *arachoides* (Burch.) Lock/ Fabaceae | Roots | BCM117179 | 250 | 250 | >500 |
| *Senna petersiana* (Bolle) Lock/ Fabaceae | Root bark | BC08 | >1000 | >1000 | 250 |
| *Sida rhombifolia* L. var. *rhombifolia*/ Malvaceae | Stems and leaves | BCM119355 | 1000 | >1000 | >500 |

| | | | | | |
|---|---|---|---|---|---|
| *Solanum tomentosum* L./ Solanaceae | Roots and fruit | BCM117177 | >1000 | 500 | >500 |
| *Sphedamnocarpus pruriens* (A.Juss.) Szyszyl. var. *pruriens*/ Malpighiaceae | Roots | BCM119342 | >1000 | 125 | 250 |
| *Strychnos madagascariensis* Poir/ Loganiaceae | Root bark | BCM117163 | >1000 | NT | >500 |
| *Synadenium cupulare* L.C.Wheeler/ Euphorbiaceae | Roots | BC109 | >1000 | NT | >500 |
| *Terminalia sericea* Burch. ex DC./ Combretaceae | Fruit/stems/leaves | BCM118704 | 500 | >1000 | >500 |
| *Trichilia emetica* Vahl./ Meliaceae | Root bark | BCM119354 | >1000 | 1000 | 125 |
| *Vernonia colorata* (Willd.) Drake var. *colorata*/ Compositae | Roots | BCM117160 | NT | 500 | >500 |
| *Xerophyta retinervis* Baker/ Velloziaceae | Whole plant | BC126 | >1000 | >1000 | >500 |
| *Ximenia americana* var. *microphylla* Welw./ Olacaceae | Thorns/leaves/stems | BC114 | 500 | >1000 | 500 |
| *Ximenia caffra* Sond. var. *caffra*/ Olacaceae | Roots | BCM119346 | >1000 | 1000 | >500 |
| *Ziziphus mucronata* Willd. var. *mucronata*/ Rhamnaceae | Leaves/stems/thorns | BCM117165 | >1000 | 250 | >500 |
| Positive control[b] | | | 0.3125 | 0.98 | 0.78 |

[a] Minimum inhibitory concentration; [b] Positive control for *Mycobacterium smegmatis* (Ciproflaxin), *Mycobacterium tuberculosis* (Isoniazid), and for *Propionibacterium acnes* (Tetracycline); NT – Not tested due to insufficient amount of extract.

## MATERIALS AND METHODS

*Bacterial strains and cell lines*

The A375 cell line was donated by the University of Johannesburg, Department of Biochemistry, Johannesburg. *Propionibacterium acnes* (ATCC 11287) was purchased from Anatech Analytical Technology (Johannesburg, SA). *Mycobacterium tuberculosis* (H37Rv), in MGIT media, and *Mycobacterium smegmatis* ($MC^2$ 155) was donated by the Department of Medical Microbiology, University of Pretoria.

*Chemical and reagents*

Recombinant mycothiol disulfide reductase (Mtr) was prepared as previously described[7]. L-epinephrine, hematin porcine, arachidonic acid, ibuprofen, human recombinant cyclooxygenase-2 (COX2), glutathione reductase (Gtr), molecular grade water, NADPH, Dimethyl-sulfoxide (DMSO) and Ellman's (DTNB) reagent were obtained from Sigma-Aldrich (St. Louis, MO, USA). Anaerocult jars and anaerocult A strips were purchased from Merck (Pty) Ltd. All other chemicals and reagents were of analytical grade and were acquired from Sigma Chemicals Co. (St. Louis, MO, USA).

*Culture medium and antibiotics*

Cell culture materials and reagents such as, fetal bovine serum (FBS), medium, and antibiotics were supplied by Highveld Biological (Pty) Ltd. (Modderfontein, Johannesburg, RSA). Middlebrook 7H9 Broth, Middlebrook OADC (Oleic Albumin Dextrose Catalase) growth supplement and Middlebrook 7H11 agar base were obtained from Sigma-Aldrich (St. Louis, MO, USA). The PANTA plus antibiotic mixture was obtained from BD Biosciences (Heidelberg, Germany). Nutrient agar and nutrient broth were purchased from Merck (Pty) Ltd.

*Kits and cell viability reagents*

The $PGE_2$ EIA kit was purchased from Enzo Life Sciences (MI, USA). The Cell Proliferation Kit II (XTT) was purchased from Roche Diagnostics (Pty) Ltd. (Randburg, Johannesburg, RSA). PrestoBlue was purchased from Life Technologies (Johannesburg, RSA) and Alamar Blue was purchased from Thermofisher Scientific Inc. (Waltham, MA, USA).

*Plant collection*

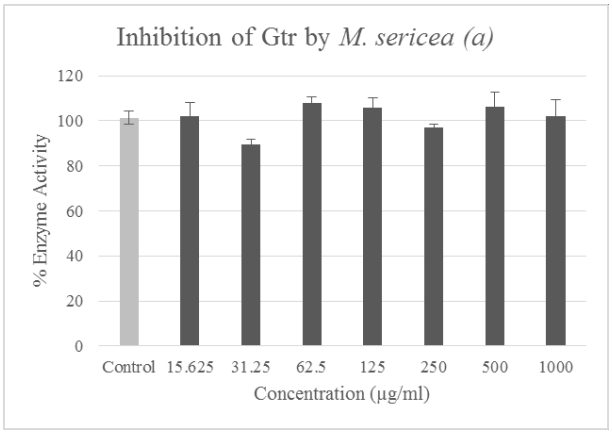
Plant material was collected from different villages within the Jongilanga community, Mpumalanga. Herbarium specimens were prepared and deposited into the HGWJ Schweickerdt Herbarium (Pru) of the University of Pretoria. Each plant species was provided with a specimen number (Table 1). Herbarium specimens were taxonomically identified at the HGWJ Schweickert Herbarium of the University of Pretoria and the South African National Biodiversity Institute.

*Plant extraction*

The plant material was shade dried for two weeks and then ground to a fine powder. The dried powder of each plant (20 g) was macerated in distilled ethanol (300 ml) and extracted for 48 h (twice) on a shaker. The plants were then filtered using a Buchner funnel with Whatman No.1 filter paper. The filtrate of each plant was collected and subjected to reduced pressure using a Büchi Rotavapor R-200. The extracts were kept in a cold room until further use. All plant names have been checked using The Plant List www.theplantlist.org

*Cell culture and cytotoxicity*

The human malignant melanoma (A375) cell line was maintained in culture flasks containing Dulbecco's Modified Eagles Medium (DMEM). The cells were supplemented with 1% antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin and 250 µg/l fungizone) and 10% heat-inactivated fetal bovine serum. The cells were grown at 37°C and 5% $CO_2$ and sub-cultured after cells formed an 80% confluent monolayer.



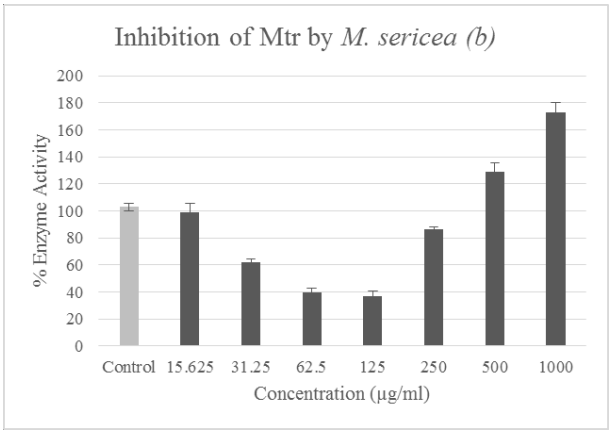




Figure 1: a) Glutathione reductase activity exposed to various concentrations of *M. sericea*; b) Mycothiol disulfide reductase activity exposed to various concentrations of *M. sericea*. Data is represented as Mean ± SD, n=3, ANOVA p-value <0.05, * p-value <0.05, ** p-value <0.001 when compared to the control (+).

Table 2: Anti-inflammatory and antioxidant and activity of *M. sericea.*

| Sample | COX-2[a] inhibition $IC_{50}$[b] in µg/ml | DPPH[c] inhibition $IC_{50}$ in µg/ml |
|---|---|---|
| *Mundulea sericea* | 10.70±1.14 | 60.52±2.40 |
| Ibuprofen[d] | 0.13±0.02 | - |
| Vitamin C[e] | - | 2.47±0.34 |

[a]Cyclooxygenase-2; [b]Fifty percent inhibitory concentration; [c]1,1-Diphenyl-2-picryl-hydrazyl; [d]Positive control for COX-2 inhibition; [e]Positive control for antioxidant activity.

Cytotoxicity was measured using the 2, 3-Bis-(2-methoxy-4-nitro-5-sulfophenyl]- 2H-tetrazolium-5-carboxyanilide salt (XTT) method. The method described by Berrington and Lall was used[8]. One hundred microliters of cells were seeded in 96-well plates at $1\times10^5$ cells/ml and incubated for 24 h at 37ºC and 5% $CO_2$ for cells to adhere. The extracts were prepared at a stock solution of 20 mg/ml. Serial dilutions were prepared to final concentrations ranging from 3.12-400 µg/ml, to screen for activity, and further incubated for 72 h. Controls included vehicle treated cells (2% DMSO), medium and cells, and a positive control, 'Actinomycin D' with concentrations ranging between 0.002 - 0.5 µg/ml. Blank plates were included as above without any cells. After 72 h, XTT (50 µl) was added to a final concentration of 0.3 mg/ml and the plates were further incubated for 2 h. The absorbance was read at 490 nm and 690 nm (reference wavelength) using a BIO-TEK Power-Wave XS multiwell plate reader (A.D.P, Weltevreden Park, South Africa). The assay was performed in triplicate to calculate a range in which the fifty percent inhibitory concentration ($IC_{50}$) could be determined.

*Antimicrobial activity against P. acnes*

The antimicrobial activity was performed according to Eloff (1998), with slight modifications[9]. *P. acnes* were maintained on sterile nutrient agar plates and sub-cultured. Subcultures of 72 h were inoculated in nutrient broth and treated to determine the minimum inhibitory concentration (MIC). Extracts were dissolved in 10% DMSO. In 96-well plates, 100 µl of each extract was serially diluted 2-fold (in triplicate) with concentration ranging from 3.9 – 500 µg/ml. *P. acnes* subcultures were inoculated in sterile nutrient broth and prepared to a density of $1.5 \times 10^8$ colony forming units (CFU) per ml (CFU/ml) corresponding with the 0.5 McFarland Standard. Inoculated nutrient broth (100 µl) was added to the plates. Tetracycline (0.2 mg/ml), at a concentration ranging from 0.781 – 100 µg/ml, and 10% DMSO were used as the positive and negative controls, respectively. After 72 h incubation at 37°C in anaerobic jars (containing anaerocult A), 20 µl of PrestoBlue was added as the indicator of bacterial growth. The MIC was assessed 1 h after the addition of the growth indicators, as the lowest concentration that inhibited bacterial growth.

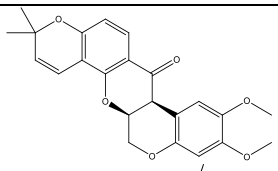
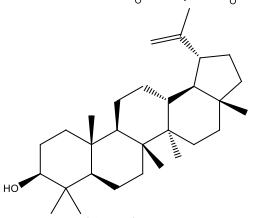
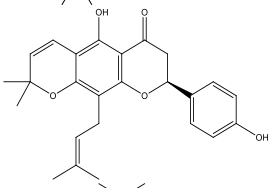
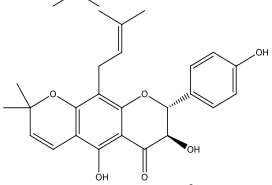
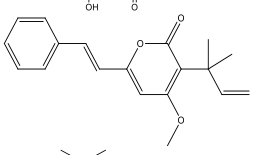
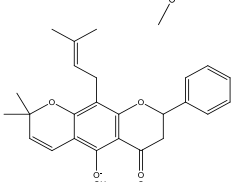
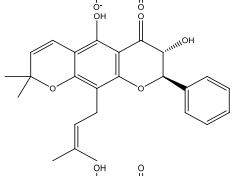
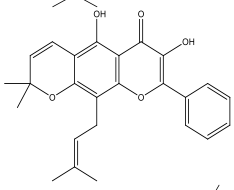
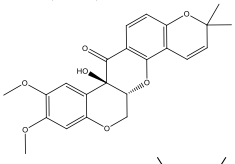
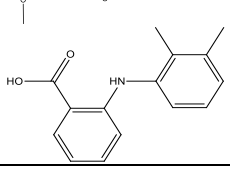
*Antimycobacterial activity*

*M. smegmatis* is a fast growing and non-pathogenic species of mycobacterium. It is most commonly used as a model in the physiology of mycobacteria, as it has relevance to the pathogenic species *M. tuberculosis*[10].

*M. tuberculosis (H37Rv)*

*M. tuberculosis* was prepared two weeks before the commencement of the MABA assay. The bacterium was sub-cultured and incubated at 37ºC using Middlebrook 7H9 broth that was supplemented with glycerol, PANTA and OADC enrichment for 21 days. The inoculum was prepared in sterile 7H9 medium adjusted to a 0.5 McFarland standard ($1.5 \times 10^8$ CFU/ml). This concentration was further diluted to 1:20 ratio and used throughout the experiment. The MIC of the extracts was determined according to the method of Franzblau et al., (1998) with slight modifications[11]. The ethanol extracts were dissolved in 20% DMSO in sterile Middlebrook 7H9 media to obtain a stock solution of 200 mg/ml. A 2-fold serial dilution of each extract was made with the 7H9 medium to yield volumes of 100 µl/well and final concentrations ranging from 3.13 – 1000 µg/ml. Isoniazid (INH), at final concentrations ranging from 0.03 - 4.0 µg/ml served as the positive control. The inoculum (100 µl) was added to each well to make up the final volume of 200 µl. Control wells, without the tested extracts, and a 5%

Table 3: Docking Score of compounds from *M. sericea* with respect to the COX-2 enzyme.

| Compound | Structure | Goldscore COX-2 | Residues involved in H-bond interactions |
|---|---|---|---|
| Deguelin |  | 33.52 | Arg120, Tyr385, Ser530 |
| Lupeol |  | - | - |
| Lupinifolin |  | 30.98 | - |
| Lupinifolinol |  | 50.36 | Arg120, Tyr355, Ser530 |
| Mundulea lactone |  | 45.84 | - |
| Mundulin |  | 53.09 | Ser530 |
| Mundulinol |  | 33.39 | - |
| Sericetin |  | 28.43 | Ser530 |
| Tephrosin |  | 46.20 | Arg120, Tyr355, Tyr385, Ser530 |
| Mefenamic acid |  | 56.27 | Tyr385, Ser530 |

DMSO solvent control, were also included in the assay. The outer wells contained 200 µl sterile distilled water to compensate for evaporation. The plates were sealed and incubated at 37ºC for 5 days. Forty microliters of 1:1 Alamar Blue reagent: 10% Tween 80 was added to the

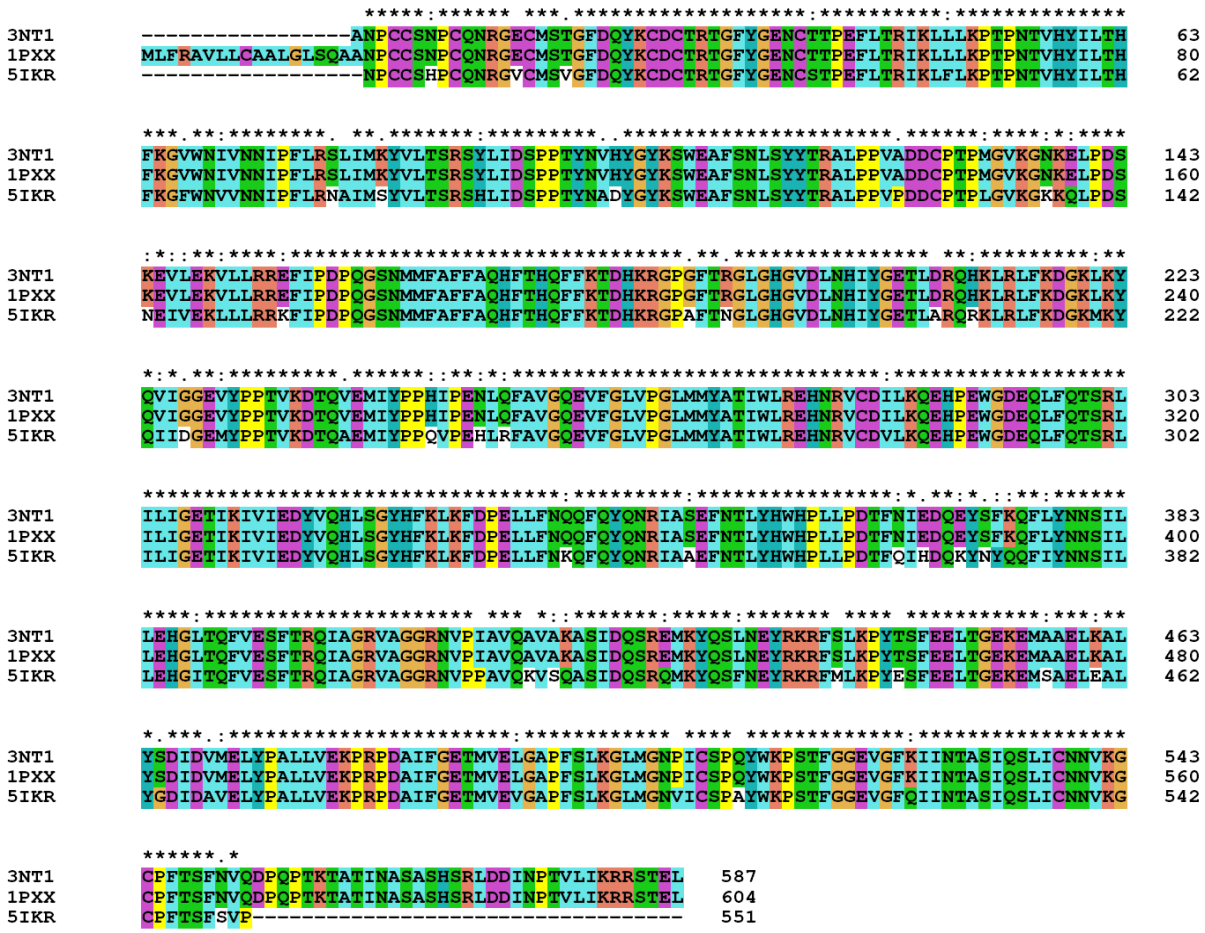



Figure 2: Multiple sequence alignment of COX-2 enzymes from *Mus musculus* (PDB ID: 3NT1, 1PXX) and *Homo sapiens* (PDB ID: 5IKR).

medium control wells, and re-incubated at 37ºC for 24 h. After observing a colour change, Alamar blue reagent was added to the rest of the test wells, and incubated at 37ºC for an additional 24 h. A colour change from blue to pink was indicative of bacterial growth.

*M. smegmatis (MC² 155)*

*M. smegmatis* was cultured in Middlebrook 7H9 broth medium, supplemented with Tween 80, and grown for 24 h at 37ºC. The bacteria were sub-cultured and incubated for a further 24 h at 37ºC or until an optical density ($OD_{600}$) of 0.1 was obtained. Stock concentrations of the extracts were prepared in 20% DMSO at 4000 µg/ml and Ciprofloxacin was prepared in sterile distilled water at 20 µg/ml. One hundred microliters of the extracts and controls were added to the top wells of a 96-well plate, in triplicate. Controls included 5% DMSO, Ciprofloxacin, media only and bacteria only. Serial dilutions were prepared to final concentrations ranging from 7.8 - 1000 µg/ml and 0.04 - 5 µg/ml for the extracts and Ciprofloxacin respectively. One hundred microliters of the prepared *M. smegmatis* culture was added to each well containing the diluted samples to obtain a final volume of 200 µl. The plates were incubated at 37°C for 24 h. After incubation, 50 µl of 1:1 Alamar Blue: 10% Tween 80 was added to the wells. The plates were incubated for a further 24 h at 37°C, and the colour change was recorded.

Having observed the highest activity for *Mundulea sericea* against A375 cells, *P. acnes*, *M. tuberculosis* and *M. smegmatis* it was decided to determine the inhibitory activity of the extract on the DPPH free radical, the COX-2 inflammatory enzyme as well as the affinity of the extract for Gtr and Mtr (Table 1 & 2).

*Enzyme kinetics on Mycothiol disulfide and Glutathione reductase*

An adapted method of Hamilton et al., (2009) was used to determine the enzyme inhibition potential of the *M. sericea* extract on glutathione (Gtr) and mycothiol disulfide (Mtr) reductases[7]. Briefly, the inhibition assay with Mtr and Gtr were carried out at 35ºC in a 96-well plate with 50 mM Hepes (pH 7.6), 0.1 mM EDTA, NADPH (70 µM), Ellman's reagent (DTNB) (100 µM), substrate GSSG and MSSM (60 µM) and varying concentrations of the extract. Stock concentrations of the extract were dissolved in DMSO at a final concentration of 10 mg/ml. The final assay volume was 200 µl. Mtr and Gtr were pre-incubated with NADPH for 5 min at 30ºC before initiating the reaction by the addition of the substrate. Enzyme activity was monitored by means of the increase in absorbance at 405 nm due to TNB formation. The kinetic read intervals were set to capture data every 15 s and the assay was left to run for 15 min at a set temperature of $35^0$C. Initial rates were measured from the linear region of the progress

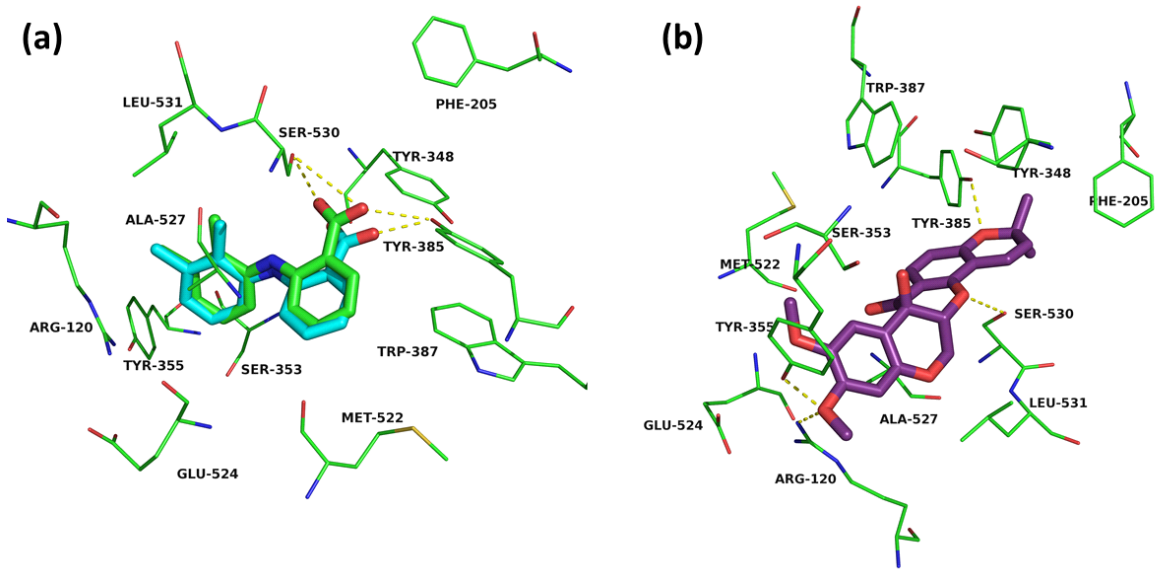



Figure 3: (a) Comparison of the docking poses of co-crystal ligand and re-docked mefenamic acid showing very little difference in the RMSD. (b) Docking pose of tephrosin showing moderate docking Gold score.

curve. The percentage inhibition was calculated according to the 100% activity where no inhibitor was present.

*DPPH scavenging activity*

The method of Berrington and Lall (2012) was followed to determine the radical scavenging capacity (RSC) of the extract[8]. Stock solutions of Vitamin C and the *M. sericea* extract were prepared at 2 mg/ml and 10 mg/ml respectively. To each well in the top row of a 96-well plate, 200 µl of distilled water was added. To the rest of the wells, 110 µl of distilled water was added as a medium. Twenty microliters of extract was added to the first top wells, in triplicate, followed by serial dilution. Final concentrations of the extract and Vitamin C ranged from 3.9 - 500 µg/ml and 0.781 – 100 µg/ml respectively. Ethanol was used as the blank control. Lastly, 90 µl of 40 mM 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) ethanolic solution was added to each well, except for the negative control wells where distilled water was added. The plate was left to develop, covered in aluminium foil for 30 min. Absorbencies were determined using a BIO-TEK Power-Wave XS multi-plate reader at a wavelength of 515 nm, using KC junior software.

*Cyclooxygenase-2 assay*

The assay was performed as described by Reininger and Bauer (2006) using human recombinant COX-2[12]. COX-2 (0.5 units/ reaction) was added to 180 µl of 100 mM TRIS buffer (pH 8.0), 5 µM porcine hematin, 18 mM L-epinephrine, and 50 µM $Na_2EDTA$ in a 96-well plate. Stock concentrations of the extracts were prepared at 10 mg/ml in DMSO. Ten microlitres of the extracts were added to the wells at a final concentration of 10 µg/ml. Ibuprofen was used as the positive control and tested at final concentrations of 10 µM (stock concentration), 2 µM and 0.4 µM. DMSO at 5 % was used as the vehicle control. The reaction was initiated after 5 min incubation at room temperature by adding 5 µl of 10 µM arachidonic acid to all the wells and incubated for a further 20 min. Lastly, 10 µl of 10% formic acid was added to stop the reaction. The amount of $PGE_2$ produced was measured using the $PGE_2$ ELISA kit after the dilution of samples in a ratio of 1:15. The absorbance was read at 405 nm using a BIO-TEK Power-Wave XS multi-well plate reader. The results were expressed as percentage inhibition of $PGE_2$ synthesis in comparison with the blank. The $IC_{50}$ values were calculated from four different concentrations using Microsoft Excel.

*Molecular docking of COX-2*

Molecular docking was performed using the molecular docking program GOLD[13]. It uses a genetic algorithm which considers ligand conformational flexibility along with partial protein flexibility i.e. side chain residues. The default docking parameters were employed for the docking study. It includes 100,000 genetic operations on a population size of 100 individuals and mutation rate of 95 as used by various recent studies[14–16]. The crystal structure of COX-2 from *Homo sapiens* was taken from the Protein Data Bank (PDB ID: 5IKR)[17]. It has a crystal structure resolution of 2.34Å and contained an inhibitor; mefenamic acid in the active site. The structures of the compounds used for docking into the COX-2 crystal structure were sketched using Chemdraw3D and minimized considering RMSD cut-off of 0.1Å. The docking protocol was set by extracting and re-docking mefenamic acid in the COX-2 crystal structure with RMSD ≤ 0.68Å. It was followed by docking of all compounds including the known inhibitor, mefenamic acid, into the active site defined as 6Å regions around the co-crystal ligand in the COX-2 enzyme. Further, all docked compounds were evaluated for possible molecular interactions with COX-2 active site residues using PyMol Molecular Graphics System[18].

*Statistical analysis*

The presented data is expressed as the mean ± SD (n=3). Statistical analysis was done using one-way analysis of variance (ANOVA) using the GraphPad Prism statistical software. An ANOVA with post Tukey's comparison was

used for the Gtr and Mtr assay with difference $p<0.001$ as statistically significant.

## RESULTS AND DISCUSSION

*Plant collection*

In the present study, 74 plant extracts (total of 33 families) were prepared to determine their anticancer and antimicrobial activities. Some extracts were prepared from the same plant species, however, a different plant part was extracted. The majority of plant species collected form part of the Fabaceae family (19%). Thereafter, Combretaceae (5.4%), Euphorbiaceae (5.4%), Acanthaceae (5.4%), and Asteraceae (4.1%) together form a total of 39.3%. The remaining 60.7% belong to 28 families, with the majority represented by one plant species; however, there are some that are also represented by two species (Table 1). This indicates that traditionally the Fabaceae family forms the majority of plants that are used for medicinal purposes (Table 1). Plant extracts were prepared from 11 different plant parts; of which the majority was prepared from roots (28%), followed by leaves (23%) and stems (18%). The root bark and bulbs each contributed towards 6.3%, followed by aerial parts, seeds, and the whole plant, each contributing 3.6%. Lastly, flowers and bark each made up 2.7% and fruit extracted the least with only 1.8%.

*Cytotoxicity on A375 cells*

All the extracts were tested for their anticancer activity against human melanoma cells (A375). The only extract which showed potential against the selected cell line was the ethanolic root extract of *M. sericea*, which belongs to the Fabaceae family, with an $IC_{50}$ value ranging between 50 and 100 µg/ml. This was compared to the positive control, Actinomycin D with an $IC_{50}$ value of $3.5 \times 10^{-2} \pm 0.001$ µg/ml. The cytotoxicity of *M. sericea* is not well documented however, there are many reports on the cytotoxicity of *Mundulea chapelieri*. In a previous study by Cao et al., (2004), 11 compounds were isolated from the methanolic extract of *M. chapelieri*, namely: isomundulinol, 3-deoxy-MS-II, 8-(3, 3-dimethylallyl)-5, 7-dimethoxyflavanone, MS-II, mudulinol, mundulone, munetone, rotenolone, rotenone, tephrosin, and 8-alpha-acetoxyelemol. The isolated compounds were then tested on human ovarian cancer cells (A2780) and rotenolone and rotenone were found to be the most active with $IC_{50}$ values of 0.5 and 0.7 µg/ml respectively.

In a study by Mazimba et al., (2012) similar compounds were isolated from the leaves, stem bark and twigs of *M. sericea*[19]. This included 11 known compounds and 1 new compound. Compounds present included; mundulea lactone, mundulin, lupinifolin, MS-II, mundulinol, lupinifolinol. 5-methoxylupinifolinol, sericetin, hexacosanyl tetracosanoate, α-stigmasterol, lupeol and a new compound 5-methoxymundulin.

There are reports on the anticancer activity of a compound present in *M. sericea*, known as deguelin. It has been reported to significantly inhibit the proliferation of various breast cancer cell lines, lung cancer and prostate cancer[20–22].

*Antibacterial activity against P. acnes*

The most predominant plant family used in this study was the Fabaceae family with a total number of 14 species. It was not surprising that most of the active species against *P. acnes* came from this plant family as they are used in the treatment of a number skin disorders, including wound healing, infectious diseases, sores or ulcers, skin irritation, burns and inflammatory skin conditions[23]. *Dalbergia melanoxylon* and *M. sericea* showed the lowest MICs against *P. acnes* (Table 1). An ethanolic extract of *D. melanoxylon* previously showed antimicrobial activity against *Staphylococcus aureus*, a Gram-positive microorganism known to cause skin disorders, with an MIC of 49 µg/ml[24]. The non-polar extracts of *M. sericea* have also been tested against *S. aureus* where the bark showed an MIC of 10 µg/ml while both the twigs and leaves showed an MIC of 50 µg/ml[19]. In the present study, the roots of *Dichrostachys cinerea* had an MIC of 125 µg/ml and showed better activity compared to the leaves and pods with an MIC of 500 µg/ml against *P. acnes* in a study conducted by Sharma and Lall, (2014)[25]. *Acacia karroo* inhibited *P. acnes* at a concentration of 125 µg/ml. This species also showed an MIC of 125 µg/ml when extracted with acetone[26]. *Chamaecrista capensis* had an MIC of 62.5 µg/ml in the present study, and is reported for the first time for its antibacterial activity against *P. acnes*.

The other prominent plants investigated belonged to the Combretaceae and Euphorbiaceae families. There were no active extracts from the Euphorbiaceae, however, *Combretum apiculatum* and *Combretum imberbe* from the Combretaceae family showed MICs of 125 µg/ml and 31.25 µg/ml, respectively against *P. acnes*. These results were confirmed by a study performed by Sharma and Lall (2014) where the MIC of *C. apiculatum* against *P. acnes* strain 11827 was also found to be 125 µg/ml[25]. There have been many reports on the antibacterial activity of *C. imberbe* against other bacteria by previous researchers. The antimicrobial activity of the dichloromethane (DCM) leaf extract of *C. imberbe* showed an MIC of 39 µg/ml against *S. aureus*. The DCM extract was then fractionated to obtain a chloroform partition which showed an MIC of 10 µg/ml. From this partition, 5 oleanene-type triterpenoids were obtained. These included 1, 3-Dihydroxy-12-oleanen-29-oic, 1-Hydroxy-12-olean-30-oic acid, 3, 30-Dihydroxyl-12-oleanen-22-one, 1, 3, 24-Trihydroxyl-12-olean-29-oic acid and 1, 23-Dihydroxy-12-oleanen-29-oic-acid-3-O-2,4-di-acetyl-l-rhamnopyranoside which showed MICs at 125, 94, 125, 63 and 63 µg/ml, respectively[27,28]. The acetone leaf extract of *C. imberbe* showed an MIC of 1600 µg/ml and that of *C. apiculatum* 400 µg/ml against *S. aureus*. This suggested that increasing the polarity of the extraction solvent might decrease the antimicrobial activity against Gram-positive bacteria as reported earlier[29]. *P. acnes* was also more susceptible to antibiotics when compared to other *Staphylococcus* species such as *S. epidermidis* which have been isolated from acne lesions. This could explain why the MICs against *P. acnes* in this study are lower than those found in other studies which were tested against *S. aureus*[30].

*Antimycobacterial activity*

The general trend observed for antimycobacterial activity in the present study was that the MIC obtained against *M. smegmatis* was found to be lower than against *M. tuberculosis* (Table 1). This may be due to the differences between the two species of mycobacteria. The Fabaceae and Combretaceae families showed the most promising activity against both the species of mycobacteria. Fabaceae/ Leguminosae are the second biggest plant family and regarded as one of the most important due to its edibility and medicinal properties.

Many plant extracts investigated within this family showed significant inhibition of both *M. smegmatis* and *M. tuberculosis*. *M. sericea* showed the highest inhibition against both *M. tuberculosis* and *M. smegmatis* with MIC values of 125 µg/ml and 31.25 µg/ml respectively. *Crotalaria agatiflora* and *Indigofera arrecta* also showed promising results with MIC values of 250 and 500 µg/ml against *M. smegmatis* and *M. tuberculosis* respectively. Many of the phytochemicals responsible for antimycobacterial activity are flavonoids, saponins and tannins[31,32]. Scrophulariaceae has a family name that can be traced back to the word scrofula, a tuberculosis infection of the lymph nodes in the neck. Many plants in this family are known to be antiviral, anti-inflammatory, antifungal and antimicrobial. Phytochemical analysis showed iridoids and saponins as the main constituents responsible for its medicinal properties[32]. Although only moderate activity was found for *Asparagus buchananii* against *M. tuberculosis* with an MIC of 500 µg/ml, Asparagaceae contains alkaloids known for their antimicrobial properties[33]. *Blepharis subvolubilis* subsp. s*ubvolubilis* from the Acanthaceae family showed an MIC of 250 µg/ml against *M. smegmatis.* Three out of the four plant extracts tested from the Combretaceae family showed antimycobacterial activity against both mycobacteria. *C. imberbe* showed the lowest MIC values against both *M. smegmatis* and *M. tuberculosis* with MIC values of 250 µg/ml and 125 µg/ml respectively. Combretaceae is known for its antibacterial properties and many species of this family have been used traditionally for treating individuals infected with tuberculosis[34]. Numerous compounds known for their antimicrobial properties have been isolated from this family. Many plant species found in the Commelinaceae family have been reported for their antioxidant and antibacterial properties. *Commelina benghalensis* showed activity against *M. tuberculosis* with an MIC of 250 µg/ml.

A known active compound against mycobacteria found in the Ebenaceae family is 7-methyljuglone (7-Mj)[35]. This compound is found in both the *Diospyros* and *Euclea* genus as well as in *Diospyros lycioides*, which may be the reason for the MIC values of 500 µg/ml and 250 µg/ml found against *M. smegmatis* and *M. tuberculosis*, respectively[36]. Asteraceae, Verbenaceae, Cactaceae, Apocynaceae, Solanaceae, Compositae and Rhamnaceae all have moderate activity against *M. tuberculosis* with MIC values of between 250 and 500 µg/ml. Oleaceae and Sapindaceae also had moderate activity against *M. smegmatis* with MIC values of 500 µg/ml and high MIC values of 1000 µg/ml. *Sphedamnocarpus pruriens* subsp. *pruriens* showed high activity against *M. tuberculosis* with an MIC value of 125 µg/ml. No previous results were found for activity against any mycobacteria species.

*Enzyme inhibition on Mycothiol disulfide and Glutathione reductase*

Most living organisms contain a thiol group, and a functional homolog of Glutathione has been identified from *M. tuberculosis*. This low molecular weight thiol producing enzyme was named mycothiol disulfide reductase (Mtr)[37]. The enzyme catalyzes the reduction of mycothiol disulfide to mycothiol (MSH)[38]. Like its glutathione human analogue, MSH plays a crucial role in protection from oxidative stress within the bacteria and is vital for the survival of the bacteria[39]. Bacteria deficient in producing MSH have shown increased sensitivity to oxidative stress, highlighting the importance of this process Mtr as a possible drug target[40].

Inhibition assays of *M. sericea* on human glutathione (Gtr) and mycobacterial mycothiol disulfide reductase (Mtr) were evaluated. The extract of *M. sericea* showed no inhibition on Gtr at a concentration range of 15.62 – 1000 µg/ml (Fig.1a). The activity on Mtr was more substantial with inhibition occurring at 62.5 and 125 µg/ml (Fig.1b). An increase in activity could be observed at 1000 µg/ml, this might be explained by solvent saturation and micro-precipitation of the dissolved compounds. A slight precipitation could be observed at the highest concentration, which will have an effect on the scattering of light due to the turbidity

Antioxidant activity of Mundulea sericea

The ethanolic stem extract of *M. sericea* in the present study showed a dose-dependent inhibitory effect on the DPPH free radical which was compared to that of the positive control Vitamin C with an $IC_{50}$ of 60.52 ± 2.40 µg/ml (Table 2). The antioxidant activity of *M. sericea* is not well explored as no previous reports were found on its activity. There are however, reports that detected the presence of flavonoids and terpenoids in both the leaves and shoots of *M. sericea*, which explains the marginal antioxidant activity as observed in the present study[41].

Table 2: Anti-inflammatory and antioxidant and activity of *M. sericea*

*Anti-inflammatory activity of Mundulea sericea*

The anti-inflammatory activity of the *M. sericea* extract was determined by using the COX-2 enzyme. The extract was able to inhibit the COX-2 enzyme, which was comparable to that of the positive control, Ibuprofen (Table 2). In a previous study by Lee et al., (2004), the rotenoid, deguelin, which has previously been isolated from *M. sericea* was able to inhibit COX-2 protein expression in squamous human bronchial epithelia cells (HBE)[42].

*Molecular docking of COX-2*

The molecular docking study was performed to determine the binding mode of compounds isolated from *M. sericea* in the COX-2 active site. The compounds previously described by Mazimba et al., (2012) and Bester and Grobler (2008) were used for docking purposes (Table 3)[19,43]. Previous molecular docking studies have been performed using the COX-2 crystal structures (PDB

ID: 3NT1, 1PXX) from *Mus musculus*[44–47]. In these studies, active site residues Ser353, Tyr355, Tyr385, Arg513, Met522, and Ser530 were reported to be involved in the H-bond interactions with docked compounds. However, H-bond interactions with residues Tyr355, Tyr385 and Ser530 were reported to be key interactions for COX-2 inhibition.

In the present study, the COX-2 crystal structure (PDB ID: 5IKR) from *Homo sapiens* were used for the docking study. It has an 88% amino acid sequence identity with the COX-2 enzyme from *Mus musculus* (Fig.3). Furthermore, a fitness score termed as Gold docking score was used to rank the various docked poses of the compounds. The docking scores were derived using the geometrical properties and bonding affinities of the ligand. All compounds were docked in the active site of COX-2 successfully.

The mefenamic acid inhibitor was present as a co-crystal ligand in the 5IKR structure. It was docked into the active site of COX-2 showing very little deviation from the co-crystal ligand (RMSD $\leq$ 0.68Å). In addition, similar to previous reports, re-docked mefenamic acid also showed key H-bond interactions with active site residues Tyr385 and Ser530 for COX-2 inhibition (Fig 4).

Among the docked compounds, lupinifolinol and mundulin showed high docking scores of 50.3 and 53.09 respectively and formed H-bond interactions with the active site residues. In contrast lupinifolin, mundulea lactone and mundulinol did not show any H-bond interactions with the active site residues therefore, the docking scores were lower compared to lupinifolinol and mundulin. Lupeol, having a five fused ring, did not dock into the active site whereas tephrosin, with a five fused ring system, showed a docking score of 46.20. It showed that fused saturated rings produced conformational restriction on lupeol, however, the three unsaturated rings in tephrosin provided sufficient planarity to allow accommodation in the active site. The rest of the compounds showed reasonable docking scores and interactions with the key residues along with some additional residues in the cavity. The observation can be concluded as cumulative interactions of these compounds may be responsible for COX-2 inhibitory activity of *M. sericea*.

## CONCLUSION

*M. sericea* was able to inhibit the growth of A375 cells, as well as the two species of mycobacteria and *P. acnes*. These results show the broad spectrum activity of the ethanolic extract of *M. sericea*. The extract also showed potential as an anti-inflammatory and its affinity for Mtr as a target for the inhibition of *M. tuberculosis*. Furthermore, based on molecular docking studies, lupinifolinol and mundulin showed the highest fit for the COX-2 enzyme when compared to mefenamic acid. These two compounds could potentially explain the anti-inflammatory activity of *M. sericea*.

## ACKNOWLEDGEMENT

This study was made possible by the generous funding of Mr Christian Courtin-Clarins, Chairman of the Clarins Group. The authors also wish to acknowledge the National Research Foundation for the financial support. The SA Medical Research Council for providing the facilities to perform the antimycobacterial assays against *M. tuberculosis* as well as Braidens from the Jongilanga community for his technical assistance.

## CONFLICT OF INTEREST

The authors declare that there are not conflicts of interest.

## REFERENCES

1. Mander M, Ntuli L, Diederichs N, Mavundla K. Economics of the traditional medicine trade in South Africa: health care delivery. South African Heal Rev [Internet] 2007;189–96. Available from: http://www.hst.org.za/uploads/files/chap13_07.pdf
2. CANSA. Fact Sheet - Skin Cancer 2010 [Internet]. 2010 [cited 2016 Mar 23]; Available from: http://www.cansa.org.za/files/2012/05/SKIN_CANCER_Leaflet-2010.pdf
3. Hartshorne ST. Dermatological disorders in Johannesburg, South Africa. Clin Exp Dermatol 2003;28(6):661–5.
4. Dlova NC, Mankahla A, Madala N, Grobler A, Tsoka-Gwegweni J, Hift RJ. The spectrum of skin diseases in a black population in Durban, KwaZulu-Natal, South Africa. Int J Dermatol 2015;54(3):279–85.
5. WHO. Global tuberculosis report 2014 (WHO/HTM/TB/2014.08). 2014.
6. Stats SA. Mortality and causes of death in South Africa. Findings from death notification [Internet]. 2014 [cited 2017 Jan 26]; Available from: www.statssa.gov.za/publications/P03093/P030932013.pdf
7. Hamilton CJ, Finlay RMJ, Stewart MJG, Bonner A. Mycothiol disulfide reductase: a continuous assay for slow time-dependent inhibitors. Anal Biochem 2009;388(1):91–6.
8. Berrington D, Lall N. Anticancer activity of certain herbs and spices on the cervical epithelial carcinoma (HeLa) cell line. Evidence-Based Complement Altern Med 2012;2012.
9. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 1998;64(8):711–3.
10. He Z, De Buck J. Cell wall proteome analysis of Mycobacterium smegmatis strain MC2 155. BMC Microbiol 2010;10(1):121.
11. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, et al. Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar Blue assay. J Clin Microbiol 1998;36(2):362–6.
12. Reininger EA, Bauer R. Prostaglandin-H-synthase (PGHS)-1 and-2 microtiter assays for the testing of herbal drugs and in vitro inhibition of PGHS-isoenzyms by polyunsaturated fatty acids from Platycodi radix. Phytomedicine 2006;13(3):164–9.
13. Verdonk ML, Cole JC, Hartshorn MJ, Murray CW, Taylor RD. Improved protein--ligand docking using

GOLD. Proteins Struct Funct Bioinforma 2003;52(4):609–23.
14. Kumar V, Sobhia ME. Molecular dynamics-based investigation of InhA substrate binding loop for diverse biological activity of direct InhA inhibitors. J Biomol Struct Dyn 2016;34(11):2434–52.
15. Lall N, Kumar V, Meyer D, Gasa N, Hamilton C, Matsabisa M, et al. In vitro and In vivo antimycobacterial, hepatoprotective and immunomodulatory activity of Euclea natalensis and its mode of action. J Ethnopharmacol 2016;194:740–8.
16. Lall N, Mogapi E, de Canha MN, Crampton B, Nqephe M, Hussein AA, et al. Insights into tyrosinase inhibition by compounds isolated from Greyia radlkoferi Szyszyl using biological activity, molecular docking and gene expression analysis. Bioorg Med Chem 2016;24(22):5953–9.
17. Bernstein FC, Koetzle TF, Williams GJB, Meyer EF, Brice MD, Rodgers JR, et al. The Protein Data Bank: a computer-based archival file for macromolecular structures. Arch Biochem Biophys 1978;185(2):584–91.
18. DeLano LW. The PyMOL molecular graphics system. 2002;
19. Mazimba O, Masesane IB, Majinda RRT, Muzila A. GC-MS analysis and antimicrobial activities of the non-polar extracts of Mundulea sericea. South African J Chem 2012;65:50–2.
20. Mehta R, Katta H, Alimirah F, Patel R, Murillo G, Peng X, et al. Deguelin action involves c-Met and EGFR signaling pathways in triple negative breast cancer cells. PLoS One 2013;8(6):e65113.
21. Zhao H, Jiao Y, Zhang Z. Deguelin inhibits the migration and invasion of lung cancer A549 and H460 cells via regulating actin cytoskeleton rearrangement. Int J Clin Exp Pathol 2015;8(12):15582.
22. Thamilselvan V, Menon M, Thamilselvan S. Anticancer efficacy of deguelin in human prostate cancer cells targeting glycogen synthase kinase-3 $β$/$β$-catenin pathway. Int J Cancer 2011;129(12):2916–27.
23. Mabona U, Van Vuuren SF. Southern African medicinal plants used to treat skin diseases. South African J Bot 2013;87:175–93.
24. Gundidza M, Gaza N. Antimicrobial activity of Dalbergia melanoxylon extracts. J Ethnopharmacol 1993;40(2):127–30.
25. Sharma R, Lall N. Antibacterial, antioxidant activities and cytotoxicity of plants against Propionibacterium acnes. S Afr J Sci 2014;110(11–12):1–8.
26. Madureira AM, Ramalhete C, Mulhovo S, Duarte A, Ferreira M-JU. Antibacterial activity of some African medicinal plants used traditionally against infectious diseases. Pharm Biol 2012;50(4):481–9.
27. Angeh JE, Huang X, Sattler I, Swan GE, Dahse H, Härtl A, et al. Antimicrobial and anti-inflammatory activity of four known and one new triterpenoid from Combretum imberbe (Combretaceae). J Ethnopharmacol 2007;110(1):56–60.
28. Eloff JN, Katerere DR, McGaw LJ. The biological activity and chemistry of the southern African Combretaceae. J Ethnopharmacol 2008;119(3):686–99.
29. Eloff JN. The antibacterial activity of 27 southern African members of the Combretaceae. S Afr J Sci 1999;95(3):148–52.
30. Nishijima S, Kurokawa I, Katoh N, Watanabe K. The bacteriology of acne vulgaris and antimicrobial susceptibility of Propionibacterium acnes and Staphylococcus epidermidis isolated from acne lesions. J Dermatol 2000;27(5):318–23.
31. Mariita R, Ogol C, Oguge N, Okemo P. Antitubercular and phytochemical investigation of methanol extracts of medicinal plants used by the Samburu community in Kenya. Trop J Pharm Res 2010;9(4).
32. McCarthy E, O'Mahony JM. What's in a name? Can Mullein Weed beat TB where modern drugs are failing? Evidence-Based Complement Altern Med 2010;2011.
33. Pallant CA, Steenkamp V. In-vitro bioactivity of Venda medicinal plants used in the treatment of respiratory conditions. Hum Exp Toxicol 2008;27(11):859–66.
34. Magwenzi R, Nyakunu C, Mukanganyama S. The Effect of Selected Combretum Species from Zimbabwe on the Growth and Drug Efflux Systems of Mycobacterium aurum and Mycobacterium smegmatis. J Microb Biochem Technol 2014;2014.
35. Mbaveng AT, Kuete V. Review of the chemistry and pharmacology of 7-methyljugulone. Afr Health Sci 2014;14(1):201–5.
36. Cai L, Wei G-X, Van Der Bijl P, Wu CD. Namibian chewing stick, Diospyros lycioides, contains antibacterial compounds against oral pathogens. J Agric Food Chem 2000;48(3):909–14.
37. Newton GL, Fahey RC. Mycothiol biochemistry. Arch Microbiol [Internet] 2002 [cited 2015 Feb 20];178(6):388–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12420157
38. Patel MP, Blanchard JS. Expression, purification, and characterization of Mycobacterium tuberculosis mycothione reductase. Biochemistry 1999;38(36):11827–33.
39. Rawat M, Newton GL, Ko M, Martinez GJ, Fahey RC, Av-Gay Y. Mycothiol-deficient Mycobacterium smegmatis mutants are hypersensitive to alkylating agents, free radicals, and antibiotics. Antimicrob Agents Chemother 2002;46(11):3348–55.
40. Sareen D, Newton GL, Fahey RC, Buchmeier NA. Mycothiol is essential for growth of Mycobacterium tuberculosis Erdman. J Bacteriol 2003;185(22):6736–40.
41. Nafuka SN, Mumbengegwi DR. Phytochemical analysis and in vitro anti-plasmodial activity of selected ethnomedicinal plants used to treat malaria associated symptoms in northern Namibia. 2013;
42. Lee H-Y, Suh Y-A, Kosmeder JW, Pezzuto JM, Hong WK, Kurie JM. Deguelin-induced inhibition of cyclooxygenase-2 expression in human bronchial epithelial cells. Clin cancer Res 2004;10(3):1074–9.

43. Bester S, Grobler A. Mundulea sericea (Willd.) A. Chev. [Internet]. 2008 [cited 2017 Jan 26];Available from: https://www.plantzafrica.com/plantklm/munduleasericea.htm
44. Gautam R, Jachak SM, Kumar V, Mohan CG. Synthesis, biological evaluation and molecular docking studies of stellatin derivatives as cyclooxygenase (COX-1, COX-2) inhibitors and anti-inflammatory agents. Bioorg Med Chem Lett 2011;21(6):1612–6.
45. Grover J, Kumar V, Sobhia ME, Jachak SM. Synthesis, biological evaluation and docking analysis of 3-methyl-1-phenylchromeno [4, 3-c] pyrazol-4 (1H)-ones as potential cyclooxygenase-2 (COX-2) inhibitors. Bioorg Med Chem Lett 2014;24(19):4638–42.
46. Grover J, Bhatt N, Kumar V, Patel NK, Gondaliya BJ, Sobhia ME, et al. 2, 5-Diaryl-1, 3, 4-oxadiazoles as selective COX-2 inhibitors and anti-inflammatory agents. RSC Adv 2015;5(56):45535–44.
47. Grover J, Kumar V, Singh V, Bairwa K, Sobhia ME, Jachak SM. Synthesis, biological evaluation, molecular docking and theoretical evaluation of ADMET properties of nepodin and chrysophanol derivatives as potential cyclooxygenase (COX-1, COX-2) inhibitors. Eur J Med Chem 2014;80:47–56.