South African Journal of Botany 118 (2018) 138–143

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

The effects of seasonal debarking on physical structure, polyphenolic content and antibacterial and antioxidant activities of *Sclerocarya birrea* in the Nylsvley nature reserve

M. Nndwammbi [a], M.H. Ligavha-Mbelengwa [a,*], C.P. Anokwuru [b], I.D.I. Ramaite [b]

[a] Department of Botany, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa
[b] Department of Chemistry, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa

ARTICLE INFO

Article history:
Received 1 February 2018
Received in revised form 13 June 2018
Accepted 22 June 2018
Available online xxxx

Edited by K Balkwill

Keywords:
Biological activities
Seasonal debarking
Flavonoids
Phenolic acids
Sclerocarya birrea
Seasonal variation

ABSTRACT

Continuous debarking results in the death of medicinal trees. The aim of this study was to evaluate the effects of seasonal debarking on the physical structure, polyphenolic content and biological activities of *Sclerocarya birrea* at Nylsvley Nature Reserve in Limpopo Province, South Africa. Bark samples were collected from trees during the autumn, winter, spring and summer seasons. During each visit the created scars were examined for any physical changes. The effect of debarking on the polyphenolic content, antioxidant and antibacterial activities of methanol extracts of the powdered bark samples was determined. Seasonal debarking resulted in infestation by insects, reduction in callus formation and decay of stems. The results also indicated that seasonal debarking did not affect the polyphenolic concentration. However, debarking significantly ($p < 0.05$) increased the DPPH antioxidant activity of the experimental trees (IC_{50} 21.30 ± 0.62 μg/mL) compared to the control (IC_{50} 46.0 ± 0.8 μg/mL). Seasonal debarking also increased the antibacterial activities of the experimental trees compared to the control against the selected pathogens. A further relatively long-term study is required to conclusively establish the effect of seasonal debarking on the bioactive constituents.

1. Introduction

Since time immemorial, plants have been exploited as key sources for medication, as they have been found to be reservoirs of chemical compounds (Saxena et al., 2013). "Approximately 80% of the African population uses traditional plants to deal with health problems" (Tsobou et al., 2016). Tree bark is widely used for medicinal purposes in village communities (Delvaux et al., 2013), and thus, the use of bark is fundamental to traditional health care in many countries (Pasztory et al., 2016). Threats posed to plant species are due to the disappearance of indigenous knowledge concerning methods of collecting plant materials, as well as their uses and unsustainable harvesting (Delvaux et al., 2009).

Plants depend on their physical structure and chemical profile for defense (Ngubeni, 2015). Bark comprises the outer bark rhytidome (dead tissue) separated from the inner tissues by cork layers, which restrict the free passage of pathogens, insects and any other form of destruction, and the live tissue called the phloem constitutes the inner bark (Lev-Yadun, 2011). The inner bark is the connecting organ between the leaf and root. Debarking refers to the removal of the bark of a plant (Williams et al., 2007) and it interferes with the exchange of materials, and can lead to the death of the tree (Delvaux et al., 2010). Recovery patterns and survival of trees after debarking is dependent on intensity of debarking, season of debarking, and size of the tree (Delvaux et al., 2010). Unsustainable utilization of plants, especially medicinal plants species, has become an issue that requires urgent attention in South Africa (Williams et al., 2013).

Sclerocarya birrea (A. Rich.) Hochst. is a member of the Anacardiaceae family that is commonly known as Marula and is an indigenous, deciduous savanna tree that grows to a height of 20 m (Russo et al., 2013). The stem has a rough gray outer bark that forms narrow openings (Mariod and Abdelwahab, 2012). The inner bark is red, pink or yellowish with darker stripes (Mojeremane and Tshwenyane, 2004). The bark has

Abbreviations: $AlCl_3$, aluminum chloride; ATCC, America type culture collection; ANOVA, analysis of variance; CDPPH, control 2,2-diphenyl-1-picrylhydrazyl; CRP, control reducing power; CTFC, control total flavonoid content; CTPC, control total phenolic content; °C, degree Celsius; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDPPH, experimental 2,2-diphenyl-1-picrylhydrazyl; ERP, experimental reducing power; ETFC, experimental total flavonoid content; ETPC, experimental total phenolic content; $FeCl_3$, ferric chloride; g, gram; h, hour; IC_{50}, 50% Inhibitory concentration; INT, iodo-nitro tetrazolium; km, kilometer; m, meter; MR, methicillin resistant; MS, methicillin susceptible; μg/ml, microgram per milliliter; μL, microliter; mgGAE/g, milligram gallic acid equivalent per gram; mg/mL, milligram per milliliter; mgQE/g, milligram quercetin equivalent per gram; mL, milliliter; MIC, minimum inhibitory concentration; Min, minutes; M, molar; nm, nanometer; %, percentage; $K_3Fe\,[CN]_6$, potassium ferricyanide; Na_2CO_3, sodium carbonate; TFC, total flavonoid content; TPC, total phenolic content; TCA, tricholo acetic acid; UV, ultra violet.

* Corresponding author.
E-mail address: maanda.ligavha@univen.ac.za (M.H. Ligavha-Mbelengwa).

https://doi.org/10.1016/j.sajb.2018.06.018
0254-6299/

medicinal value and is used alone or mixed with bark from other species to treat ailments such as dysentery, hepatitis, diseases associated with blood circulation and diarrhea, amongst others (Kutama et al., 2013). About one-third of the plant materials used in South African traditional medicine come from the bark of trees (Grace et al., 2002). Studies on recovery of tree bark (Delvaux et al., 2013) and survival (Helm et al., 2009) have been reported. However, there are limited reports on the effect of debarking on the chemical constituents of the bark materials used in herbal preparations. The aim of this study is to provide the first report on the effect of seasonal debarking on physical structure, polyphenolic content and antibacterial and antioxidant activities of *S. birrea*. *S. birrea* is a protected species that is widespread. In some protected areas, such as Kruger National Park (Seloana et al., 2017) and Nylsvley Nature Reserve, its population size can be limited by animals that browse the seedlings while large animals such as elephants damage the stem, sometimes breaking it (Helm and Witkowski, 2012). In most parts of Africa, the species is protected in communal areas under jurisdictions of the local chief (Mabala, 2017), thus restricting cutting of the species in homesteads and agricultural areas.

2. Materials and methods

2.1. Study area

The study was conducted in the savanna biome at Nylsvley Nature Reserve, located at 24° 39′ 17″ South and 28° 41′ 28″ East in the Waterberg region of the Limpopo Province, South Africa. The reserve is situated 12 km south of Mookgopong (Limpopo Department of Economic Development, Environmental and Tourism, 2013). The reserve is climatically classified as semi-arid, experiencing hot wet summer and cool dry winter season each year (Tooth et al., 2002). The vegetation is clustered into two, namely: mixed bushveld and a clay thorn bushveld, differing in accordance with soil type and water regime (Dippenaar-Schoeman et al., 2009).

2.2. Study design

S. birrea trees were examined for any scars and three unwounded individuals, in the same population, were selected as the experimental group. A total of 12 trees was used as the control, three trees during each season. Debarking was carried out within one year, in the four seasons starting from autumn. In the experimental group, a single scar was made during each season, making a total of four scars on the same tree throughout the study period. In the control group, only a single scar was made on each set of trees, throughout the study period. The removed bark included the periderm and rhytidome up to secondary phloem, but the vascular cambium, secondary xylem, sapwood, xylem rays and heartwood were left. Square wounds, one third of the size of the circumference of each individual tree were inflicted using an ax, saw and chisel. For experimental individuals, successive injuries of the same size were made during each visit, on the same tree. Physical scars were observed after every three months after incision for a period of one year. The collected bark was stored inside a cooler box containing ice to inactivate enzymes and then taken to the laboratory for drying and preparation for extraction. During each visit, the created scars were observed for any physical changes, including callus formation and gum exudation.

2.3. Preparation of extracts

Collected bark was cut into small pieces using a knife and scissors, air dried for 4 weeks and ground with an industrial grinder (Dietz-moteren KG, Deltingen unter Teck, Germany) into powder and stored in brown envelopes until needed for extraction. From each sample, 50 g of powder was soaked in 500 mL of methanol with continuous shaking for 72 h followed by filtration with Whatman filter paper (No 4). The residues were discarded. The methanol filtrates were evaporated under reduced pressure at 40 °C using a Rota vapor (Buchi, Swizerland). The dried samples were weighed and stored in a refrigerator at 4 °C until further use.

2.4. Determination of the content of polyphenol compounds

A stock solution of 10 mg/mL in Dimethyl sulfoxide (DMSO) was prepared for each sample.

2.4.1. Determination of total phenolic content

Total phenolic content of *S. birrea* bark extracts was determined according to the Folin–Ciocalteu method as reported by Anokwuru et al. (2017). The 96-well plate was filled with 80 μL of water and then 20 μL of each sample was added in triplicate. Twenty microliters of 10% Folin–Ciocalteu reagent and 60 μL of 7% sodium carbonate (Na_2CO_3) were added to the mixture respectively. Prior to reading, 120 μL of distilled water was added to the mixture and allowed to stand for 30 min. The absorbance was read at 760 nm using a microplate reader (Versa Max, China). A gallic acid standard curve was used to convert the absorbance of the extracts to total phenolic content expressed in milligram gallic acid equivalent per gram of the extract (mg GAE/g).

2.4.2. Determination of total flavonoid content

Flavonoid content of *S. birrea* stem bark extracts was determined according to the aluminum chloride method reported by Olajuyigbe and Afolayan (2011). A volume of 100 μL of 2% aluminum chloride ($AlCl_3$) — ethanol was added to 100 μL of each extract in a 96-well plate. Tests were carried out in triplicate. The mixture was allowed to stand for 1 h, and then the absorbance was measured with a microplate reader (Versa Max, China) at 420 nm. A quercetin standard curve was used to convert the absorbance of the extracts to total phenolic content expressed in milligrams quercetin equivalent per gram of the extract (mg QE/g).

2.5. Antioxidant activity

For antioxidant and reducing power, the volume of the extracts was 10 mg/mL of DMSO.

2.5.1. Free radical scavenging activity

The DPPH free radical scavenging activity was evaluated according to Anokwuru et al. (2017). A volume of 200 μL of 0.3 M DPPH (2,2-diphenyl-1-picrylhydrazyl) was added in all the wells containing 100 μL of serially diluted sample or standard solution. The mixture was allowed to stand in darkness for 30 min. The absorbance was read with a microplate reader at 517 nm. The percentage of radical scavenging was calculated using the following formula:

$$\%\text{free radical scavenging activity} = \frac{\text{Absorbance of DPPH} - \text{Absorbance of sample}}{\text{Absorbance of DPPH}} \times 100$$

The highest IC_{50} value designates the lowest antioxidant activity while the lowest IC_{50} values specify the highest activity.

2.5.2. Reducing power activity

The reducing power activity of the extracts was determined as described by Anokwuru et al. (2017). A volume of 50 μL of 0.2 M sodium phosphate buffer was added into all 96-well plates containing 50 μL of serially diluted samples or standard (gallic acid; quercetin or ascorbic acid). The mixture was incubated for 20 min at 50 °C after adding 50 μL of 1% potassium ferricyanide [K_3Fe [CN]$_6$] in each well. After incubation, 50 μL of 10% Trichloro Acetic acid (TCA) was added to each well. Then 80 μL of the mixture was extracted and poured into a new well plate. It