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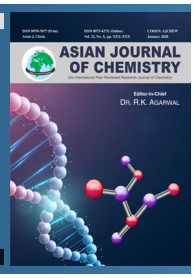

Seasonal Variations of Phytochemical Content and Antioxidant Activity of *Senna italica* Leaves

SIBUSISO C. MNCUBE, SECHENE S. GOLOLO* and MOTETELO A. MOGALE

Department of Biochemistry, Sefako Makgatho Health Sciences University, Ga-Rankuwa (Pretoria), South Africa

*Corresponding author: Tel: +2712 521 3242; E-mail: Stanley.gololo@smu.ac.za

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Senna italica is a widely used medicinal plant well known for their laxative properties and high antioxidant activity, as well as possession of a variety of bioactive compounds. For its optimum usage, the current study was aimed at the determination of seasonal variations in the phytochemical content and antioxidant activity of its leaves. The leaves of *Senna italica* were collected during each of the four seasons (autumn, winter, spring, summer) in the year 2018. Total phenolic, total tannin, total flavonoid and total saponin contents of the leaves were determined using UV spectrophotometric method. Antioxidant activity was evaluated using the DPPH free radical scavenging activity and H_2O_2 reducing activity assays. Total phenolic content was recorded in higher amounts during autumn; total tannin content was in higher amounts during autumn and summer; total flavonoids were higher during autumn and summer and total saponins were higher in summer. High antioxidant activity strength demonstrated through relatively lower IC_{50} was shown in summer and autumn which, was consistent with the relative phytochemical contents. The current results suggest that the optimum period of harvest for maximum benefit regarding the antioxidant activity of *Senna italica* leaves is between summer and autumn and that its antioxidant activity strength is aligned to the accumulation of the antioxidant compounds.

Keywords: *Senna italica* leaves, Seasonal variations, Phytochemical content, Antioxidant activity, DPPH assay, H_2O_2 assay.

INTRODUCTION

Oxidative stress is implicated in the formation of many adverse health events in the human body. Imbalanced accumulation of free radicals in the body contributes to oxidative stress that leads to DNA and tissue damage, as well as high levels of inflammation. More often, DNA and tissue damage culminate into a number of health disorders such as rapid ageing, Alzheimer's disease, Parkinson disease and neurodegenerative diseases [1]. Antioxidants play a major role in the maintenance of balanced free radicals present in the body and therefore, help mitigate the development of diseases associated with high levels of free radicals. Antioxidants also act as anti-ageing and anticancer agents, as well as against neurodegenerative diseases [2].

An antioxidant can be regarded as a molecule that has the capacity to slow or prevent the oxidation of other molecules [3]. Antioxidant activity is one of the most studied biological activities inherent to medicinal plants. Many medicinal plants contain large amounts of antioxidant phytochemicals such as polyphenols, which play a role in adsorbing and neutralizing free radicals [4]. Antioxidants are known to interrupt the free radical chain of oxidation by donating hydrogen from phenolic hydroxyl groups and to form stable products, which do not initiate or propagate further oxidation of lipids [5]. There are several methods reported for the evaluation of the antioxidant activity of medicinal plants. The most frequently utilized methods for the determination of the antioxidant activity of medicinal plants extracts are 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the hydrogen peroxide (H_2O_2) assay. The DPPH assay is a simple, cost-effective and widely inclusive to solid and liquid samples that indicate the hydrogen-donating capacity and hence the antioxidant activity of medicinal plants extracts [6]. The H_2O_2 assay is used to determine the electron-donating activity of plants extracts components for mitigation against reactive oxygen species (ROS) [7].

Although the body is able to maintain the free radical balance through its self-generated antioxidant agents, often free radicals are produced in excess to the available body antioxidants. As such, there is a need to supplement the body with

antioxidants from other sources either than the human body [8]. Medicinal plants have proven to be reliable sources for supplementary antioxidants. Extracts of plants have been shown to possess different classes of phytochemicals with antioxidant properties [9]. However, the accumulation of phytochemicals in different plants parts is affected by variations in growing conditions such as seasonal variations and differences in altitudes of the geographical location [10]. Therefore, for effective usage of medicinal plants, it is important to determine the growing conditions that afford higher phytochemical content and optimum biological activities that contribute to their medicinal value.

Senna italica is one medicinal plant with wide usage in traditional medicinal systems of many communities of the world. The leaves of the plant species, well known for its laxative properties, is reported to possess important phytochemicals that include phenolic, tannin, flavonoid and saponin compounds, as well as many medicinal activities such as antioxidant, anticancer, antidiabetic and anti-inflammatory activities [11]. Our observation has indicated that the leaves of plant species are available for collection during different seasons of the year in the Limpopo province of South Africa, although with notable differences in morphological quality. To determine the suitable harvesting period for the optimum usage of the leaves of *Senna italica* in traditional medicine, seasonal variations in the phytochemical content and antioxidant activity was investigated.

EXPERIMENTAL

Sample collection, preparation and extraction: The leaves of *Senna italica* were collected from the Zebediela subregion in the Capricorn district in Limpopo province (South Africa) during four different seasons of the year (autumn, winter, spring and summer). The collected leaves were left to dry off at room temperature and ground into powder and then stored in a dark place until used. Then the powdered plant material (5 g) was extracted with 50 mL of *n*-hexane, dichloromethane, acetone and methanol in a serial sequential exhaustive extraction procedure and the resultant extracts were filtered, dried under the stream of air and stored in the dark until further usage.

Determination of phytochemical contents

Determination of total phenols: Total phenols were determined through the Folin-Ciocalteu method as described by Abdille *et al.* [12], with some slight modifications. Standard solutions of gallic acid (20; 40; 60; 80 and 100 mg/mL) were prepared. An amount of 5 mL from each gallic acid solution was transferred into a test tube containing 5 mL of distilled water. Following this procedure, 0.5 mL of Folin-Ciocalteu phenol reagent was added to the test tubes and the reaction mixture was thoroughly shaken. Then 5 mL of sodium carbonate (Na_2CO_3, 7%) was added and the reaction was incubated at room temperature for 90 min. After incubation, aliquots of about 300 µL of the reaction mixture solutions were transferred into a 96 well microplate and absorbance was measured at 550 nm using a SpectraMax ID 96 well microplate reader (Separations, South Africa). The same procedure was followed with *n*-hexane, dichloromethane, acetone and methanol extracts of *S. italica* leaves collected during different seasons.

Determination of total tannins: Total tannins were determined through the Folin-Ciocalteu method as described by Abdille *et al.* [12], with some slight modifications. Standard solutions of gallic acid (20; 40; 60; 80 and 100 mg/mL) were prepared. An amount of 5 mL from each gallic acid solution was transferred into a test tube containing 5 mL of distilled water. Then 0.5 mL of each gallic acid solution was added to a 50 mL volumetric flask containing 37.5 mL of distilled water 2.5 mL of the Folin-Ciocalteu phenol reagent and 1 mL of 35% Na_2CO_3. The reaction mixture was shaken thoroughly and kept at room temperature for 30 min. After incubation, aliquots of about 300 µL of the reaction mixture solutions were transferred into a 96 well microplate and absorbance was measured at 725 nm using a SpectraMax ID 96 well microplate reader (Separations, South Africa). The same procedure was followed with *n*-hexane, dichloromethane, acetone and methanol extracts of *S. italica* leaves collected during different seasons.

Determination of total flavonoids: Total flavonoids were determined using the aluminium chloride method as described by Ghasemzadeh *et al.* [13], with some modification. About 1 mL of each *S. italica* leaf extract was mixed with 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 1 M of 0.2 potassium acetate and 5.6 mL of distilled water. The mixture was left at room temperature for 30 min. Then absorbance of the reaction mixture was measured at 420 nm using a SpectraMax ID 96 well microplate reader (Separations, South Africa). Quercetin solution was used as a control standard and the flavonoid contents of the extracts of *S. italica* leaves collected during different seasons were expressed as mg quercetin equivalence (QE)/g of the plant extracts.

Determination of total saponins: Total saponins were determined using the vanillin-sulphuric acid calorimetric reaction method as described by Makkar *et al.* [14], with slight modifications. Plant extracts aliquots (50 µL) were diluted with 250 µL of distilled water and followed by addition of 250 µL of vanillin-sulphuric acid reagent (8% of vanillin in 99% ethanol plus 5 mL of 72% sulphuric acid). The solution was incubated in a water bath at 60 °C for 10 min and was afterwards cooled in ice-cold water. The absorbance was then measured at 544 nm using a SpectraMax ID 96 well microplate reader (Separations, South Africa). Diosgenin was used as a control standard and total saponins of the extracts of *S. italica* leaves collected during different seasons were expressed as mg diosgenin equivalence (DE)/g extract.

Antioxidant activity

DPPH radical scavenging activity assay: The DPPH free radical scavenging activity of the extracts of *S. italica* leaves collected during different seasons was determined according to the method of Abdille *et al.* [12], with some slight modifications. Different concentrations (0 to 0.3 mg/mL) of the acetone and methanol extracts of *S. italica* were prepared and 150 µL of the extract solutions were transferred into different wells of a 96 well microplate. Then 50 µL of 0.1 mM methanol

TABLE-1
SEASONAL VARIATION OF THE PHYTOCHEMICAL CONTENTS IN THE LEAVES OF *S. italica*

Phytochemical type	Total phenolic (mg GAE/g)	Total tannin (mg GAE/g)	Total flavonoid (mg QE/g)	Total saponin (mg DE/g)	Total phytochemicals (mg/g extract)
Autumn	0.540 ± 0.015^{a}	1.61 ± 0.017^{a}	0.307 ± 0.007^{a}	0.124 ± 0.076^{a}	2.58^{a}
Winter	0.381 ± 0.070^{b}	1.22 ± 0.084^{b}	0.076 ± 0.061^{b}	0.081 ± 0.079^{b}	1.76^{b}
Spring	0.176 ± 0.026^{c}	1.30 ± 0.120^{b}	0.118 ± 0.013^{c}	0.122 ± 0.038^{a}	1.72^{b}
Summer	0.143 ± 0.020^{d}	1.65 ± 0.070^{a}	0.305 ± 0.011^{a}	0.358 ± 0.104^{c}	2.46^{a}

[a,b,c,d]Values with different letters in a column are significantly different and those with similar letters are not significantly different ($p < 0.05$); values are averages of triplicates.

solution of DPPH was added into the extract solutions and incubated for 30 min at room temperature. Following incubation, the absorbance of the samples was measured at 517 nm using a SpectraMax ID 96 well microplate reader (Separations, South Africa). The percentage inhibition of the extracts was calculated as follows:

$$\text{DPPH inhibition (\%)} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

where, $A_{extract}$ is the absorbance reading of the extracts or the standard sample with DPPH and $A_{control}$ the absorbance of DPPH without extracts or standard.

Hydrogen peroxide assay: The H_2O_2 scavenging activity of the extracts of *S. italica* leaves collected during different seasons was determined according to the method described by Olivier *et al.* [15] with some slight modifications. About 20 mM of hydrogen peroxide solution was prepared using phosphate buffer saline (PBS), pH 7.40. Different concentrations (0 to 0.3 mg/mL) of the acetone and methanol extracts of *S. italica* were prepared and about 150 µL of the extract solutions were transferred into different wells of a 96 well microplate. Then 50 µL of 20 mM hydrogen peroxide solution was added and followed by incubation for 10 min at room temperature. The absorbance of the reaction mixture was then measured at 560 nm using a SpectraMax ID 96 well microplate reader (Separations, South Africa) using ascorbic acid as the control standard. The percentage inhibition of the extracts was calculated as follows:

$$H_2O_2 \text{ inhibition (\%)} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

where, $A_{extract}$ is the absorbance reading of the extracts or the standard sample with DPPH and $A_{control}$ the absorbance of DPPH without extracts or standard.

RESULTS AND DISCUSSION

The extracts of *S. italica* leaves collected during the different seasons were subjected to phytochemical contents analysis for total phenolics, total tannins, total flavonoids and total saponins of which, the results are presented in Table-1. The results demonstrated the recording of higher amounts of total phenolics in autumn; total tannins in autumn and summer; total flavonoids in autumn and summer; and total saponins in summer. The acetone and methanol extracts of *S. italica* collected during varying seasons, having shown appreciable antioxidant activity during screening, were subjected to quantitative antioxidant activity analysis. All samples extracts showed DPPH and H_2O_2 inhibition in a dose-dependent manner. The antioxidant activity strength or capacity of the extracts was evaluated based on extract concentration that gave 50% inhibition (IC_{50} µg/mL) against the each of the free radicals and the results are presented in Tables 2 and 3. On average, the autumn and the summer samples showed lower IC_{50} values (25 µg/mL and 33 µg/mL, respectively) against DPPH which, compared well with that of the positive control standard, ascorbic acid (35 µg/mL). Both samples, autumn and summer samples, also showed lower IC_{50} values (19 µg/mL and 18 µg/mL, respectively) against H_2O_2 which, were similar to that of ascorbic acid (18 µg/mL).

TABLE-2
SEASONAL VARIATIONS IN THE IC_{50} (µg/mL) VALUES OF THE EXTRACTS OF *S. italica* LEAVES AGAINST DPPH

Samples	Acetone extract	Methanol extract	Average
Autumn	30	20	25^{a}
Winter	160	75	118^{b}
Spring	52	60	56^{c}
Summer	50	15	33^{d}
Ascorbic acid	35	35	35^{d}

[a,b,c,d]Values with different letters in a column are significantly different and those with similar letters are not significantly different ($p < 0.05$); values were interpolated from plots obtained from triplicate measurements.

TABLE-3
SEASONAL VARIATIONS IN THE IC_{50} (µg/mL) VALUES OF THE EXTRACTS OF *S. italica* LEAVES AGAINST H_2O_2

Samples	Acetone extract	Methanol extract	Average
Autumn	27	10	19^{a}
Winter	75	63	69^{b}
Spring	75	50	63^{b}
Summer	20	15	18^{a}
Ascorbic acid	18	18	18^{a}

[a,b,c,d]:V with different letters in a column are significantly different and those with similar letters are not significantly different ($p < 0.05$); values were interpolated from plots obtained from triplicate measurements.

In the present study, the phytochemical contents and antioxidant activity of the extracts of *S. italica* leaves collecting during varying seasons of the year were investigated. Information on the optimum season for an accumulation of phytochemicals

in plants is important in determining the suitable harvest period [16]. The results showed some seasonal variations in the phytochemical contents and antioxidant activities of the leaves of *S. italica*. In total, higher amounts of phytochemicals were recorded in the autumn and summer samples. In addition, higher antioxidant activity capacity, which was demonstrated through lower IC_{50} values against DPPH and H_2O_2, was recorded in the autumn and summer samples, which was consistent with the recorded total phytochemical contents. Generally, the harvesting of medicinal plants in South Africa usually occurs during the summer and autumn seasons due to their vegetative availability. However, according to WHO [17], the suitability of the collection time for medicinal plants should be informed by the quality and quantity of bioactive constituents and not the vegetative availability of such plants. The results of the current study showed the possession of higher amounts of investigated phytochemicals by the leaves of *S. italica* during the summer and autumn seasons which, notably also coincide with the earlier alluded general collection times in South Africa. Summer and autumn are mainly the rainy periods that are accompanied by relatively higher temperatures, whereas winter and spring are dry seasons with winter characterized by low temperatures and spring being the advent of a warmer season [18]. Many plants shed leaves in dry seasons such as winter and start flowering in spring. Although *S. italica* possesses some leaves in both winter and spring, they appear smaller and dark-greenish as compared to the relatively larger and deeply greenish leaves found in summer and autumn. This could explain the results obtained during the current study that showed lower phytochemical contents in winter and spring as there could be a withdrawal of metabolites from the likely to be senescing leaves during the dry seasons [19].

Biosynthesis and accumulation of phytochemicals in plants are known to be connected to the different roles they play in the plant [20]. Phenolic compounds, flavonoids, tannins and saponins serve plants as defense mechanism against pathogens and mitigation against adverse environmental conditions such as UV radiation and heat stress. As such, the biosynthesis and accumulation of phenolic compounds in plants is triggered mainly by external factors [21]. Therefore, the recording of higher amounts of phytochemicals in the leaves of *S. italica* during the rainy and higher temperatures conditions in summer and autumn indicate the existence of external stimuli for their accumulation during these seasons. Phenolic compounds, flavonoids, tannins and saponins are also reported to possess some antioxidant properties [22] which, explains the results obtained in the current study where higher antioxidant activity was recorded in samples that exhibited higher amounts of these phytochemicals.

Conclusion

The results of the present study showed seasonal variation in the phytochemical contents and antioxidant activity of the leaves of *S. italica*. The findings of the current study suggest that the suitable harvesting period for optimum benefits from the antioxidant properties of the leaves of *S. italica* is between summer and autumn.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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