International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 10, Issue 2, 2018

Original Article

COMPARATIVE QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF THE LEAVES OF *SENNA ITALICA* COLLECTED FROM DIFFERENT AREAS IN LIMPOPO PROVINCE, SOUTH AFRICA

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Received: 05 Oct 2017 Revised and Accepted: 21 Dec 2017

ABSTRACT

Objective: The current study was aimed at comparative quantitative phytochemical analysis of the leaves of *S. italica* collected from four districts in Limpopo province, South Africa, in order to establish whether geographical location have an effect on the accumulation of phytochemicals within the leaves of the plant species under study.

Methods: The leaves of *S. italica* were collected from four districts in Limpopo province of South Africa namely; Capricorn, Sekhukhune, Vhembe and Waterberg districts, dried, ground to powder and extracted using different organic solvents. The extracts of the leaf samples from different locations were subjected to quantitative phytochemical analysis for total phenolic content, total tannin content, total flavonoid content and total saponin content using spectrophotometric measurements. The resultant quantities were analysed for statistical differences.

Results: The leaf samples of *S. italica* from the four districts in Limpopo province showed significant differences (*$p<0.05$*) in their phytochemical quantities, with main data expressed as mean±SD. Total phenolic content was in highest amounts in leaf samples from Waterberg district compared to samples from other districts. Total tannin content was in highest amounts in the Vhembe district leaf samples compared to samples from other districts. Total flavonoid content was in highest amounts in the leaf samples from Waterberg district compared to samples from other districts. Total saponin content was in highest amounts in the Vhembe district leaf samples compared to samples from other districts.

Conclusion: The findings of the study thus suggest that geographical location has an effect on the accumulation of phytochemicals in the leaves of *S. italica*.

Keywords: Medicinal plants, Phytochemical accumulation, Geographical location, Altitude, Quantitative phytochemical analysis, *Senna italic*

DOI: http://dx.doi.org/10.22159/ijpps.2018v10i2.22950

INTRODUCTION

Worldwide, medicinal plants continue to be relied upon as solutions to health problems administered through traditional medicine practices [1, 2]. Medicinal plants also offer a new scope of research in disease treatment based on their alluded safety and accessibility properties [3]. The medicinal value of herbal remedies has been attributed to their bioactive phytochemical constituents such as flavonoids, tannins, terpenoids, alkaloids, anthraquinones, saponins and other secondary metabolites [4], which have the ability to induce biological responses in other organisms that include enzyme inhibition [5]. Some of the medicinal plants used as herbal remedies are widely distributed along different locations with varying environmental parameters such as altitudes and soil types. Many environmental factors can act singly or interact on medicinal plants to affect the productivity of its secondary metabolites.

Amongst environmental factors that can affect the phytochemical compositions of plants is the geographical location of growth [6, 7]. Geographical locations are different from each other, with others being costal and others being inland. However, to lend a better description in the differences in these locations, altitude may come in handy. Altitude is regarded as the height of the geographical location in relation to the sea level and is one of the parameters usually used in the description of geographical locations [8].

Senna italica, a member of the Fabaceae family, is one medicinal plant widely distributed in the Limpopo province of South Africa and is well known for its therapeutic properties [9-11]. However, it has not been establishing whether the geographical location has any effect on the quantity of its phytochemicals. The current study was aimed at the comparison of the quantity of phytochemicals of the leaves of *S. italica* collected from different areas in Limpopo province, South Africa.

MATERIALS AND METHODS

Plant samples collection and preparation of extracts

The leaves of *S. italica* Mill, subspecies *arachoides* (Burch) Lock (UNIN 11129) were collected from four districts in the Limpopo province of South Africa [namely; Capricorn district (Bolahlakgomo village, 927 m above sea level), Sekhukhune district (Apel cross village, 1420 m above sea level), Vhembe district (Mutale village, 606 m above sea level) and Waterberg district (Mosesetjane village, 1099 m above sea level)] during the summer of 2016. The districts from which the samples were collected are shown in fig. 1. The leaves were then dried at room temperature and ground to a powder using a coffee grinder (Mellerware, South Africa). The ground leaf samples (5 g) of *S. italica* were then extracted with 50 ml of *n*-hexane, dichloromethane, acetone and methanol by cold maceration using a non-sequential exhaustive extraction procedure. The resultant extracts were filtered and the solvents evaporated under a stream of air. The dried extracts were stored in the dark until further use.

Experimental procedure

The extracts of the leaf samples of *S. italica* collected from different areas were analysed and compared for total phenolic content, total flavonoid content, total tannin content and total saponin content, as described below.

Fig. 1: The map of Limpopo province indicating the four districts (Capricorn, Sekhukhune, Vhembe and Waterberg) from which samples were collected (www.capeinfo.com/blogs/wp-content/blogs.dir/akela/files/2009/05/limpopo_dlghgovza.gif)

Determination of total phenolic content

The quantity of total phenolic compounds from the leaf extracts of *S. italica* collected from four districts in the Limpopo province of South Africa was determined by the Folin-Ciocalteu assay as described by Tambe and Bhambar [12]. Standard solutions of gallic acid (20, 40, 60, 80, 100 µg/ml) were prepared from which, 1 ml of each of the gallic acid solutions was added into a 25 ml volumetric flask to create a reaction mixture. Then, 1 ml of Folin-ciocalteu phenol reagent (Sigma-Aldrich, St. Louis, Missouri, United States) was added with well shaking. After 5 min, 10 ml of 7% Sodium carbonate (Na_2CO_3) solution was added to the mixture and the volume was made up to 25 ml with distilled water. The solution was then incubated for 90 min at room temperature and the absorbance was recorded at 550 nm with an ultraviolet/visible (UV/vis) spectrophotometer (CECIL CE 1021, Cecil Instruments Limited, UK). Solutions of n-hexane, dichloromethane, acetone and methanol leaf extracts of *S. italica* were prepared and absorbance read in the same manner as described above. The total phenol content of the extracts was expressed as mg of gallic acid equivalence/g of extract (mg GAE/g).

Determination of total tannin content

The quantity of total phenolic compounds from the leaf extracts of *S. italica* collected from four districts in the Limpopo province of South Africa was determined by the Folin-Ciocalteu assay as described by Tambe and Bhambar [12]. Standard solutions of gallic acid (20, 40, 60, 80, 100 µg/ml) from which, 0.5 ml of each of the different gallic acid solutions were added to a 50 ml volumetric flask to which, was added 2.5 ml of Folin-Ciocalteu phenol reagent (Sigma-Aldrich, St. Louis, Missouri, United States), 1 ml of 35% Na_2CO_3 solution and diluted to 50 ml with distilled water. The resultant solution was well shaken and kept at room temperature for 30 min. Then absorbance of the solutions was measured at 725 nm with an UV/vis spectrophotometer (CECIL CE 1021, Cecil Instruments Limited, UK). Solutions of hexane, dichloromethane, acetone and methanol leaf extracts of the *Senna italica* were prepared and absorbance read in the same manner as described above. The phenol content of these extracts was expressed as mg of gallic acid equivalence/g of extract (mg GAE/g).

Determination of total flavonoid content

Total flavonoid content was measured by the aluminium chloride colourimetric assay as described by Tambe and Bhambar [12]. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 min, 0.3 ml of 10 % aluminium chloride was mixed. After 5 min, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. Reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described above. Then absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/vis spectrophotometer (CECIL CE 1021, Cecil Instruments Limited, UK). The total flavonoid content was expressed as mg of quercetin (Sigma-Aldrich, St. Louis, Missouri, United States) equivalence/g of extract (mg QE/g).

Determination of total saponin content

Estimation of total saponin content was determined based on vanillin-sulphuric acid colorimetric reaction as described by Senguttuvan *et al.* [13]. About 50 µl of plant extract was added to 250 µl of distilled water. To this, about 250 µl of vanillin reagent (Sigma-Aldrich, St. Louis, Missouri, United States) (800 mg of vanillin in 10 ml of 99.5% ethanol) was added. Then 2.5 ml of 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60 °C for 10 min. After 10 min, it was cooled in ice-cold water and the absorbance was read at 544 nm with an UV/vis spectrophotometer (CECIL CE 1021, Cecil Instruments Limited, UK). The values were expressed as diosgenin (Sigma-Aldrich, St. Louis, Missouri, United States) equivalents/g extract (mg DE/g) derived from a standard curve.

Statistical analysis

The significant differences of phytochemical quantities in the leaf samples from different geographical locations were determined by analysis of variance (ANOVA) using the SPSS (version 12.00) statistical package. The difference in phytochemical quantity values was considered as statistically significant at $p<0.05$, main data were expressed as mean±SD.

RESULTS

The extracts of the leaf samples of *S. italica* from four districts in Limpopo province of South Africa were analyzed for total phenolic contents, tannins, flavonoids and saponins and the results are presented in tables 1, 2, 3 and 4, respectively. The mean values of phytochemicals in different extracts of the samples were added together to obtain total values per sample from each area, for each specific phytochemical group, and statistically compared. The results showed some differences in the quantities of total phenolics, tannins, flavonoids and saponins amongst the leaf samples collected from four districts in Limpopo province.

Total phenolic content expressed as mg GAE/g extract, was found in highest amounts in leaf samples from Waterberg district compared to samples from other districts. Total tannin content also expressed as mg GAE/g extract, was in highest amounts in the Vhembe district leaf samples compared to samples from other districts. Total flavonoid content expressed as mg QE/g extract was found in highest amounts in the leaf samples from Waterberg district compared to samples from other districts. Total saponin content, expressed as mg DE/g extract was in highest amounts in the Vhembe district leaf samples compared to samples from other districts.

Table 1: **Total phenolic content (mg GAE/g extract) in the extracts of the leaf samples of *S. italica* from four districts in limpopo province, South Africa**

District	Hexane extracts	Dichloromethane extracts	Acetone extracts	Methanol extracts	Total (mg GAE/g)
Capricorn	-	0.480±0.148	0.364±0.012	1.31±0.053	2.15^{a*}
Sekhukhune	-	0.487±0.006	0.335±0.009	1.69±0.006	2.51^{b*}
Vhembe	-	0.525±0.00	0.505±0.006	2.96±0.029	3.99^{c*}
Waterberg	-	0.590±0.012	0.534±0.009	3.61±0.006	4.73^{d*}

^{a*,b*,c*,d*}: values with different alphabetical letters are significantly different, i.e., *p<0.05*; results are mean±SD with n=3 *(-: not determined)*

Table 2: **Total tannin content (mg GAE/g extract) in the extracts of the leaf samples of *S. italica* from four districts in Limpopo province, South Africa**

District	Hexane extracts	Dichloromethane extracts	Acetone extracts	Methanol extracts	Total (mg GAE/g)
Capricorn	-	-	0.767±0.285	8.23±0.235	8.99^{a*}
Sekhukhune	-	-	0.221±0.135	30.8±0.358	31.0^{b*}
Vhembe	-	-	1.09±0.223	40.7±1.48	41.8^{d*}
Waterberg	-	-	0.531±0.319	37.5±0.184	38.0^{c*}

^{a*,b*,c*,d*}: values with different alphabetical letters are significantly different, i.e., *p<0.05*; results are mean±SD with n=3 *(-: not determined)*

Table 3: **Total flavonoid content (mg QE/g extract) in the extracts of the leaf samples of *S. italica* from four districts in Limpopo province, South Africa**

District	Hexane extracts	Dichloromethane extracts	Acetone extracts	Methanol extracts	Total (mg QE/g)
Capricorn	0.862±0.030	0.962±0.053	1.32±0.054	1.17±0.123	4.31^{a*}
Sekhukhune	0.753±0.074	0.853±0.139	0.710±0.044	2.18±0.277	4.49^{a*}
Vhembe	0.584±0.108	0.684±0.159	0.949±0.133	3.19±0.066	5.41^{b*}
Waterberg	0.924±0.199	1.02±0.202	0.941±0.057	3.33±0.175	6.22^{c*}

^{a*,b*,c*,d*}: values with different alphabetical letters are significantly different, i.e., *p<0.05*; results are mean±SD with n=3

Table 4: **Total saponin content (mg DE/g extract) in the extracts of the leaf samples of *S. italica* from four districts in Limpopo province, South Africa**

District	Hexane extracts	Dichloromethane extracts	Acetone extracts	Methanol extracts	Total (mg DE/g)
Capricorn	0.167±0.008	0.235±0.008	0.063±0.364	0.232±0.051	0.697^{a*}
Sekhukhune	0.175±0.166	0.253±0.166	0.096±0.082	0.538±2.42	1.06^{b*}
Vhembe	0.321±0.194	0.394±0.194	0.164±0.194	0.637±1.84	1.52^{d*}
Waterberg	0.235±0.004	0.303±0.048	0.162±0.048	0.536±1.92	1.24^{c*}

^{a*,b*,c*,d*}: values with different alphabetical letters are significantly different, i.e., *p<0.05*; results are mean±SD with n=3

The total quantities of phenolic, tannins, flavonoids and saponins were added together to obtain the total phytochemical content of the leaf samples of *S. italica* from each of the four districts in Limpopo province, South Africa and the results are presented in table 5. The results showed that the highest amount in phytochemical content was recorded with leaf samples from the Vhembe district (52.7 mg/g extract), followed by leaf samples from Waterberg (50.2 mg/g) and Sekhukhune (39.1 mg/g) districts, respectively. The least amount of total phytochemical content was recorded with the leaf samples from the Capricorn district (16.1 mg/g). The percentages of total phenolic, tannins, flavonoids and saponins were calculated in relation with the total phytochemical content for samples from each district and the percentages were averaged to estimate the distribution pattern of phytochemicals within the leaves of *S. italica* from Limpopo province of South Africa, and the results are shown in fig. 2. The phytochemical composition of the leaves of *S. italica* from Limpopo province appears to be dominated by tannins at 72.5% on average.

Table 5: **Total phytochemical content of the leaf samples of *S. italica* from four districts in limpopo province, South Africa**

District	Total phytochemical content (mg/g extract)
Capricorn	16.1^{a*}
Sekhukhune	39.1^{b*}
Vhembe	52.7^{d*}
Waterberg	50.2^{c*}

^{a*,b*,c*,d*}: values with different alphabetical letters are significantly different, i.e., *p<0.05*; results are the sum of different total phytochemical groups of plant sample collected at a particular location.

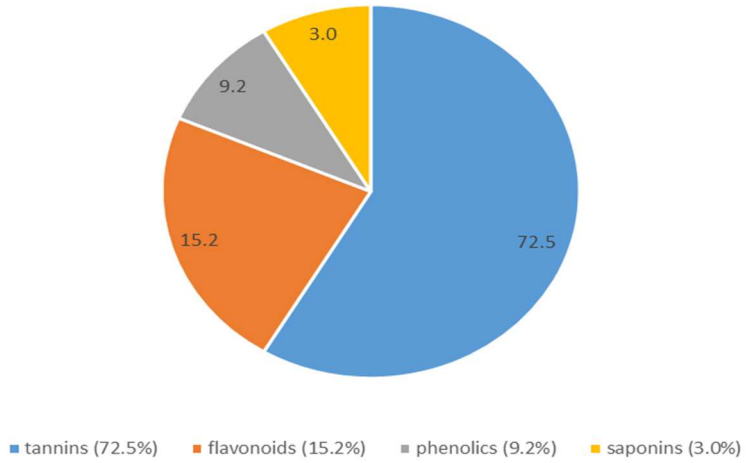

Fig. 2: The average phytochemical distribution pattern within the leaves of *S. italica* from Limpopo province, South Africa

DISCUSSION

Quantification of phytochemicals within the samples of plant parts from different locations will help in establishing whether the geographical location has an effect on the phytochemical composition of the medicinal plant under current study. It also provides knowledge on the suitable habitation area that affords optimum concentrations or quantities of specific active ingredients [14]. The dose-dependent manner has been shown in the biological activities of many Medicinal plants [15], hence the importance of harvesting medicinal plants in locations where they possess high accumulation of phytochemicals for usage in traditional medicine. In the current study, the quantities of phytochemicals within similar solvent extracts of the leaf samples of *S. italica* collected from the different areas in Limpopo province (South Africa) were found to be statistically different at $p<0.05$, suggesting that they were affected by the location conditions. However, the effect appears to be phytochemical group specific since the high accumulation of the different phytochemicals were not recorded in one location. Total phenolic and flavonoid contents were found in highest amounts in leaf samples from Waterberg district, whereas total tannin and saponin contents were found in highest amounts in samples from Vhembe district.

The addition of total phenolic, tannins, flavonoids and saponins of samples enabled the determination of the total phytochemical content of leaf samples from each district. The calculated total phytochemical content gives a clear indication of the district area with high overall accumulation of phytochemicals, and in this study leaf sample of *S. italica* from Vhembe district showed high overall amounts of phytochemicals. From the calculated total phytochemical content, the relative percentages of each phytochemical group were determined. On average, the leaf samples of *S. italica* from the four districts in Limpopo province of South Africa appear to be rich in tannins, with moderate possession of flavonoids and poor accumulation of saponins. Thus, calculation of total phytochemical content also enables the determination of the distribution pattern of phytochemicals within plant parts.

These results of the current study are in agreement with the findings of the previous study by Nchabeleng *et al.* [6], where the differences in the phytochemical composition of wild bush tea (*Athrixia phylicoides* DC) growing at locations differing in altitude was demonstrated. The four areas from which the leaf samples of *S. italica* were collected are of different altitudes and soil types. These environmental factors, altitude and soil type, were reported through previous studies to influence the accumulation of phytochemicals in medicinal plants [14, 16, 17].

CONCLUSION

The results of the current study indicate the difference in the quantities of the investigated phytochemicals of the leaf samples of *S. italica* collected from different areas in Limpopo province of South Africa. The findings of the study thus suggest that geographical location has an effect on the accumulation of phytochemicals in the leaves of *S. italica*.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Department of Biochemistry at Sefako Makgatho Health Sciences University for the facilities used to do the study and Dr Bronwyn Egan from the Department of Botany for the taxonomic authentication of the plant species. The study was financially supported through the DHET Research Development Grant (RDG: GOLOLO) funding.

AUTHORS CONTRIBUTIONS

Gololo: conceptualization of the study, data analysis and writing of the manuscript.

Mapfumari: data collection and analysis, as well as the writing of the manuscript.

Mogale: writing of the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interests

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