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Research Article

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Comparison of the TLC R_f values and UV-Visible spectral profiles of the leaf extracts of *Senna italica* collected from four districts in Limpopo province, South Africa

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ABSTRACT

Senna italica as a medicinal plant has wide usage in traditional medicine, particularly by communities in different districts of the Limpopo province, South Africa. The medicinal value of plants parts is dependent on the nature of phytochemicals they possess. The aim of the study was to obtain and compare the TLC and UV-Vis spectral profiles of S. italica collected from four districts in Limpopo province of South Africa. The leaves of S. italica were collected from four different districts (Capricorn, Sekhukhune, Vhembe and Waterburg districts) in the Limpopo province of South Africa. The dried, ground leaves were extracted with solvents of different polarities. Constituents of the different extracts were resolved on TLC plates using different solvent systems and the R_f values of resolved compound bands noted and compared between collection districts. The UV-Vis spectra of different extracts were obtained and wavelengths of compound peak signals noted and compared between collection districts. The obtained results showed in some instances a disparity on the TLC and UV-Vis spectral profiles of the leaf extracts of S. italica between collection districts. This was evidenced by the presence of compound bands at R_f values and/or presence of compound peak signals at wavelengths in other locations which were absent in other locations of occurrence within extracts obtained by same solvent. Thus, it could be concluded that the phytochemical composition of the leaves of S. italica is affected by geographical location of occurrence within Limpopo province (South Africa).

Keywords: *Senna italica*, phytochemicals, thin layer chromatography, ultraviolet-visible spectrophotometry, geographical location

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INTRODUCTION

For many years, people have been relying on plants for prevention and remedies of health challenges[1].It has been reported that about sixty percent of the South African black population still relies on medicinal plant concoctions administered by traditional healers for their health problems in preference to or in supplementation to Western medicine, particularly in rural areas [2].Plants have been shown to exert many biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, anti-atherosclerotic and antidiabetic on living systems. The biological properties of medicinal plants have been attributed to their possession of phytochemicals such as alkaloids, flavonoids, tannins, saponins, anthraquinones and terpenes [3]. Phytochemicals, are present in selected plants and are responsible for defence against pathogens, herbivores, ultra-violet (UV) radiation and are also involved in colour or pigmentation of flowers and in pollination [4;5]. Thus, plants produce phytochemicals as a

response to environmental factors that include geographic area of occurrence [6]. Geographical area of occurrence, often referred to as geographical location, may be adequately characterised based on soil type, soil pH, altitude, humidity and temperature of which have been reported to have effect on the phytochemical compositions of plants [7; 8].

S.italica belongs to the family Fabaceae and is well known for its therapeutic properties in folk medicine of some countries. A literature survey on the chemical constituents of the genus Senna revealed the presence of alkaloids, quinines and anthraquinones. These types of compounds have been isolated from heartwood, seeds, root bark, roots and leaves of genus Senna [9]. The plant species has been reported to possess substantial antibacterial activity, anti-proliferative and antioxidant properties that support its usage for the treatment of some diseases that are related to bacterial infections[10; 11]. An observation was made on the wide distribution of *S. italica* among the different districts in the Limpopo province of South Africa. The plant species is also widely used by communities in different districts of Limpopo province against a number of health complications. However, it is unknown whether the geographic area of occurrence has any effect on the phytochemical composition of *S. italica*. As such, we examined the leaves of *S. italica* collected from different districts in Limpopo province (S.A) to establish any disparity in phytochemical composition by comparing their thin layer chromatography (TLC) and ultraviolet-visible (UV-vis) spectrophotometry profiles.

EXPERIMENTAL SECTION

2.1. Plant material and soil samples

The leaves of *S. italic* Mill, subspecies *arachoides* (Burch) Lock (UNIN 11129) were collected from the four districts; namely Capricorn (Bolahlakgomo village), Sekhukhune (Apel cross village), Vhembe (Mutale village) and Waterberg(Mosesetjane village) in the Limpopo province of South Africa in February 2016. Samples were collected at afternoon in all locations. The leaves were then dried at room temperature, ground to powder and stored in the dark until used. Soil pH, soil colour, soil texture and temperatures (during collection date) of the sample collection locations were recorded.

2.2. Experimental procedure

2.2.1. *Extraction*

The ground leaves (2 g) of *S. italica* collected from different districts in Limpopo province (South Africa) were extracted with 20 ml of n-hexane, dichloromethane, acetone and methanol in a serial exhaustive extraction procedure. The resultant extracts were filtered and the solvents evaporated under a stream of air.

2.2.2. *Phytochemical analysis*

2.2.2a. *Thin Layer Chromatography*

Aliquots (10 mg/ml) of extracts were loaded on TLC plates and resolved using three different mobile phases, namely, Hexane: Ethyl acetate (9:1 v/v); Chloroform: Methanol (9:1 v/v) and Ethyl acetate: Methanol: Water (8:4:1 v/v/v). Compound bands within extracts were located and circled on TLC plates upon eye visualization, under UV light, as well as after spraying with vanillin in sulphuric acid. Circles showing compound bands were then used to calculate $R_f$values and compared between different districts.

2.2.2b.*UV-Vis spectrophotometry*

Plant extracts were dissolved with dimethylsulfoxide (DMSO) to constitute 10 mg/ml of the extracts and diluted tenfold. UV-vis spectral profiles of the diluted extracts were obtained using a Cary 50 UV-Vis spectrophotometer at Sefako Makgatho Health Sciences University. Wavelengths of peak signals depicting compound groups within extracts were noted and compared between different districts.

RESULTS

The dried ground leaves of *S. italica* collected from four districts in Limpopo province (South Africa) were sequentially extracted with n-hexane, dichloromethane, acetone and methanol. The four districts used as sample collection sites were profiled in terms of altitude, soil pH, soil colour, soil texture and temperature and recorded in Table 1. It is noted that the four locations are of different altitudes, soil pH and soil colour.

Table 1: Location parameters in the Limpopo province (South Africa) from which leaf samples of *S. italica* were collected

Location	Altitude (m)	Soil pH	Soil colour	Soil type	Temperature (February 2016)
Capricorn district (Bolahlakgomo village)	927	5.04	Red	Loamy	29°C Low 18°C 08/02/216
Sekhukhune district (Apel cross village)	1420	8.49	Light brown	Sandy	29°C Low17°C 07/02/2016
Vhembe district (Mutale village)	606	6.38	Grey	Clay	29°C Low 16°C 09/02/2016
Waterburg district (Mosesetjane village)	1099	7.71	Dark brown	Loamy	29°C Low 18°C 08/02/2016

Three mobile phases of different polarities were used for TLC analysis of the extracts and the R_f values of compound bands are reported in Table 2. Some differences were recorded in profiles of compound bands by similar extracts of samples from different districts. Emphasis was made on the compound bands (*see bold R_f values in Table 2*) from similar extracts present only in samples from one location according to mobile phases used to develop TLC chromatograms.

Lower polarity mobile phase (Hexane: Ethyl acetate, 9:1): From the hexane extracts chromatogram, compound bands with R_f values of 0.06 and 0.09 were recorded on the Capricorn district sample which were absent in samples from other districts. With the dichloromethane extracts, a compound with R_f value of 0.60 was recorded only on the Waterberg district sample. From the acetone extracts, compound bands with R_f values of 0.45 and 0.34 were recordedwith the Capricorn and Waterberg samples, respectively. From methanol extracts chromatogram, distinct compound bands with R_f values of 0.09 and 0.32 were only recorded on the Waterberg district sample. Samples from Sekhukhune and Vhembe districts did not show any distinct compound bands.

Intermediate polarity mobile phase (Chloroform: Methanol, 9:1): From the dichloromethane extracts, compound bands with R_f values of 0.17 and 0.76 were recorded on the Capricorn sample and a compound band of R_f value 0.43 was recorded on the Waterberg district. With the acetone extracts, compound bands with R_f values of 0.01 and 0.83 were recorded on the Vhembe and Waterberg districts, respectively. Methanol extracts showed the presence of distinct compound band with R_f value of 0.23 on the Sekhukhune district sample and another with R_f value of 0.87 on the Waterberg district sample. Hexane extracts from all four districts showed similar TLC profiles.

A higher polarity mobile phase (Ethyl acetate: Methanol: Water, 8:4:1), showed only few distinct compound bands amongst samples from different districts. Distinct compound bands were only recorded as follows: compound band of R_f value 0.76 with the hexane extract of the sample from the Capricorn district, compound band of R_f 0.90 with the dichloromethane extract of the sample from Waterberg district and a compound band of R_f value 0.75 with the methanol extract of sample from Waterberg district. No disparity was shown by the TLC profiles of the acetone extracts of samples from four districts.

UV-Visible spectra of the leaf extracts of *S. italica* from four districts were also obtained and are reported as peak signal wavelengths (nm) in Table 3. The results shows differences in the UV-vis spectral profiles of extracts obtained with similar solvents amongst samples collected from different district locations. Wavelengths of peak signals (*see bold values in Table 3*) from similar extracts present only in samples from one location were noted. From the hexane extracts, distinct peak signals were recorded at 420, 255, and 230 nm with the Capricorn district sample; 220 nm with the Sekhukhune district sample and 225 nm with the sample from Vhembe district. From the dichloromethane extracts, distinct peak signals were recorded at 225 and 205 nm with the Waterberg district sample only. From the acetone extracts, distinct peaks were at 245 nm for the Capricorn district sample; 255 nm for the Vhembe district sample and 280 nm for Waterberg district sample. More distinct peak signals were recorded between 300 and 350 nm from the methanol extracts of samples from Sekhukhune, Vhembe and Waterberg districts.

Table 2: Rf values showing compound bands within leaf extracts of *S. italica* from different districts in Limpopo province (South Africa). (CD- Capricorn; SD- Sekhukhune; VD- Vhembe; WD- Waterburg)

Mobile Phase: **Hexane: Ethyl Acetate (9:1): Lower polarity**															
n-Hexane extract				**Dichloromethane extract**				**Acetone extract**				**Methanol extract**			
CD	SD	VD	WD	CD	SD	VD	WD	CD	SD	VD	WD	CD	SD	VD	WD
0.06	0.10	0.11	0.10	0.04	0.10	0.11	0.06	0.13	0.13	0.13	0.13	0.11	0.11	0.12	**0.09**
0.11	0.26	0.18	0.17	0.10	0.20	0.20	0.13	**0.45**	0.41	0.39	**0.34**	0.36			0.12
0.25	0.28	0.24	0.26	0.19	0.33	0.33	0.20				0.41	0.47			**0.32**
0.29	0.33	0.29	0.29	0.28	0.54	0.54	0.29								0.39
0.33	0.37	0.34	0.33	0.34	0.89	0.89	0.33								0.47
0.37	0.48	0.37	0.37	0.39	0.97	0.99	0.38								
0.42	0.55	0.58	0.43	0.48			0.48								
0.64	0.62	0.70	0.50	0.56			0.54								
0.74	0.72	0.79	0.61	0.97			**0.60**								
0.89	0.79	0.84	0.68				0.90								
0.96	0.85	0.96	0.72				0.97								
	0.96		0.81												
			0.96												
Mobile phase:- Chloroform: Methanol (9:1): **Intermediate polarity**															
n-Hexane extracts				**Dichloromethane extracts**				**Acetone extracts**				**Methanol extracts**			
CD	SD	VD	WD	CD	SD	VD	WD	CD	SD	VD	WD	CD	SD	VD	WD
0.86	0.86	0.84	0.87	0.09	0.11	0.12	0.12	0.07	0.06	**0.01**	0.07	0.17	0.17	0.17	0.20
0.95	0.95	0.95	0.95	**0.17**	0.54	0.24	0.23	0.14	0.39	0.06	0.21	0.49	**0.23**	0.48	0.49
				0.44	0.59	0.40	0.39	0.40	0.55	0.10	0.41	0.69	0.47	0.67	0.76
				0.57	0.73	0.50	**0.43**	0.56	0.67	0.21	0.55	0.78	0.66	0.79	**0.87**
				0.67	0.83	0.54	0.49	0.71	0.96	0.39	0.71	0.96	0.78	0.98	0.99
				0.76	0.96	0.69	0.56	0.96		0.53	**0.83**		0.98		
				0.86		0.83	0.61			0.65	0.98				
				0.96		0.96	0.73			0.96					
							0.84								
							0.96								
Mobile Phase: Ethylacetate: Methanol: Water (8:4:1): Higher polarity															
n-Hexane extracts				**Dichloromethane extracts**				**Acetone extracts**				**Methanol extracts**			
CD	SD	VD	WD	CD	SD	VD	WD	CD	SD	VD	WD	CD	SD	VD	WD
0.59	0.50	0.49	0.46	0.99	0.99	0.99	**0.90**	0.52	0.88	0.77	0.56	0.07	0.08	0.07	0.08
0.76	0.59	0.57	0.54				0.99	0.78	0.97	0.81	0.78	0.36	0.41	0.38	0.35
0.90	0.69	0.70	0.68					0.84		0.89	0.83	0.55	0.54	0.54	0.55
0.96	0.96	0.95	0.88					0.89		0.95	0.89	0.66	0.67	0.64	0.70
			0.96					0.98			0.97	0.82	0.82	070	**0.75**
												0.90	0.91	0.82	0.83
												0.97	0.97	0.90	0.91
														0.95	0.96

Table 3: Peak signal wavelengths (nm) describing the UV-Vis spectra of the leaf extracts of *S. italica* collected from districts in Limpopo province. (CD: Capricorn district; SD: Sekhukhune district; VD: Vhembe district; WD: Waterberg district)

Hexane extracts				**Dichloromethane extracts**				**Acetone extracts**				**Methanol extracts**			
CD	SD	VD	WD	CD	SD	VD	WD	CD	SD	VD	WD	CD	SD	VD	WD
670	670	280	280	670	670	670	670	670	670	670	670	670	670	670	670
420	270	270	270	280	275	415	415	275	275	270	**280**	345	370	380	370
280	250	260	260	265	260	270	280	260	260	**255**	270	**290**	360	370	355
270	235	245	250	250	250	260	270	**245**	240	235	260	**265**	345	360	345
255	**220**	235	235	235	235	245	260	235	**220**	225	240	250	**335**	**350**	**330**
245	210	**225**	210	220	220	220	245	225		215	215	225	**320**	**340**	**305**
230		210					235	205		205		210	295	**325**	295
210							**225**						270	**315**	**285**
							205						260	**300**	270
													245	**280**	260
													230	270	250
													210	**255**	230
														245	**215**
														225	
														205	

DISCUSSION

Phytochemicals are very important compounds present in plant extracts that are responsible for their biological activities and as such contribute to the efficacy of the usage of plant parts in traditional medicine. Qualitative phytochemical analysis using techniques such as TLC and UV-Vis spectrophotometry is a very important step as it gives information about the nature of phytochemicals present within plant extracts. Compounds maybe characterized based on polarity using TLC and light absorption using UV-vis spectrophotometry.

The TLC profiles of same-solvent leaf extracts of *S. italica* collected from four districts in Limpopo province (South Africa) showed the presence of some compound bands of different R_f values in samples from one location that were absent in samples from other locations. UV-Vis spectral profiles of same-solvent leaf extracts also showed presence of peak signals at wavelengths in samples from one location that were absent in samples from other locations. The disparity in the TLC and UV-Vis spectral profiles displayed by extracts obtained using similar solvents amongst leaf samples collected from different districts; suggest some differences in the phytochemical composition of the leaves of *S. italic* growing at different district locations. The locations from which plant samples were collected are of different altitudes, soil pH and soil types, which is likely to have contributed to the differences in the phytochemical composition of plant samples. This is in line with previous findings that plants growing in areas with different altitudes and soil types have different phytochemical compositions [12]; [3].

CONCLUSION

Environmental factors such as altitudes, soil pH and soil types, are important in the description of the geographical locations where plants grow. The four districts (Capricorn, Sekhukhune, Vhembe and Waterberg) in Limpopo province of South Africa are of different altitudes and soil characteristics and similar extracts of *S. italica* leaves samples collected from these districts were found to have some differences in their phytochemical composition. Thus, it could be concluded that the phytochemical composition of the leaves of *S. italica* is affected by geographical location of occurrence within Limpopo province (South Africa).

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REFERENCES

[1] Verma S., Singh S.P. *Veterinary World*, **2008**, 1: 347-350
[2] Van Wyk B.E., Van Oudsthoorn B., Gericke N. Medicinal Plants of South Africa. *Briza Publications*, Pretoria, **1997**.
[3] Rauf A., Rehma W.U., Jan M.R. and Muhammad N. *Wudpecker J. Pharm. and Pharmacol.*,**2013**, 2: 021-025.
[4] Visioli F., Borsani L., Galli C. *Cardiovascular Res.*, **2000**, 47: 419-425
[5] Javanmardi J., Stushnoff C., Locke E., Vivanco J.M. *J. Food Chem.*, **2003**, 83: 547-550
[6] Van WykBE, Gericke N. People's Plants: A Guide to Useful Plants of Southern Africa. *Briza Publications*, Pretoria, **2000**.
[7] Nchabeleng L., Mudau F.N., Mariga I.K. *J. Med. Plants Res.*, **2012**, 6: 1662-1666
[8] Jayanthy A., Prakash Kumar U. and Remashree A.B. (**2013**) *Int. J. Herbal Med.*, 2013,1: 34-37.
[9] Barbosa FG, da Conceicao M, de Oliveira F, Braz-Filho R, Silveira ER. *Biochem.Sys. Ecol.*,**2004**, 32:363-365
[10] Tshikalange TE, Meyer JJM, Husein AA. *J.Ethnopharmacol.*, **2005**,96: 515–519
[11] Masoko P., Gololo S.S., Mokgotho M.P., Eloff J.N., Howard R.L. and Mampuru L.J. *Afr.J. Tr., Compl. Alt. Med*. **2010**, 7: 138-148.
[12] Ubani C.S., Oje O.A., Ihekogwo F.N.P., Eze E.A. and Okofor C.L. *Gl. Adv. Res.J. Micro.*, **2012**, 1: 120-125.