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HPLC Fingerprint Associated with Chemometrics of *Guiera senegalensis* Leaf Extracts

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ABSTRACT

Guiera senegalensis is a shrub reported to have several medicinal properties due to its vast phytochemicals. However, the concentration of these phytochemicals is affected by several factors such as location and season of harvest. The aim of this research is to establish a chromatographic fingerprint of the phytochemicals in *G. senegalensis* leaf samples collected from two states in Northwest Nigeria at two different seasons. HPLC method was adopted and validated for the fingerprint analysis of samples collected. Chemometric techniques such as percent relative standard deviation (% RSD) of the relative retention time (RRT) and relative peak area (RPA), similarity and difference analysis were conducted. The adopted HPLC method was found to be suitable for the analysis as the % RSD of both intraday and interday precisions were less than 3%. Fingerprint analysis showed excellent similarity between the samples as indicated by % RSD of RRT (< 1.6 %), degree of difference (0.00-0.014) and similarity (0.995-1) values. The high % RSD values of RPA (17-120%) indicated variations in concentrations of the phytochemicals present in the *G. senegalensis* leaf samples. This method can therefore, be used for the routine quality control of *G. senegalensis* leaf sample.

***Keywords**:* *Guiera senegalensis*, Phytochemicals, Chemometrics, Fingerprint and RP-HPLC

Introduction

Guiera senegalensis is a shrub rich in phytochemicals like catechin, myricitrin, rutin, quercetin, tetrahydroharman, harman, hyoscyamine and solanine.[1,2,3,4,5] It is traditionally used in the treatment of cough, respiratory congestion, fever, malaria, stomach pain, dysenteric diarrhea, syphilis, beriberi, leprosy and impotence.[6,7,8,9,10,11]
The type and amount of phytochemicals in a plant are to a large extent determined by method of cultivation, seasons of harvest, climate, and a host of others.[12,13,14,15] Quality control ensures the safety and efficacy of herbs as it involves the identification and quantification of their complex and variable phytochemicals.[16] Standard quality control method for the quantification of phytochemicals in herbs is difficult to achieve due to the inconsistency and complexity of the herbs.[12,13] Thus, a more reliable quality control can be achieved by determining most of the phytochemicals of herbal medicines.[12] The aim of this research is to establish a chromatographic fingerprint of the phytochemicals in *Guiera senegalensis* leaf samples collected from two states in Northwest Nigeria at two different seasons.

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Materials and Methods

Collection, identification and processing of plant materials
G. senegalensis leaf samples were collected from natural habitat in the months of January and April, 2016 from Jigawa (Dutse) and Kaduna A.B.U. Zaria) States, Northwest Nigeria and were identified with a voucher number, 1823, at the Department of Botany, Ahmadu Bello University, Zaria, Nigeria. Samples collected in the month of January were coded Fe and Dc for Jigawa and Kaduna respectively while Cc and Ac were assigned to those collected in the month of April from Jigawa and Kaduna respectively. The leaf samples were shade-dried and pulverized using mortar and pestle.

Sample preparation and fingerprint analysis
Quantities (1 g) of the air dried samples were defatted for 48 h using 10 mL hexane with occasional shaking. The solutions were then filtered and the marc expressed. Each of the expressed marc was extracted with 10 mL chloroform for 48 h with occasional shaking and then filtered. The filtrates were evaporated to dryness, using rotary evaporator, under room temperature. Portions (100 mg) of the extracts were dissolved in methanol (10 mL), filtered and analysed using the optimized chromatographic conditions. Before sample injection, the mobile phase was degassed using an online degasser and the base line was allowed to equilibrate for at least 10 minutes. Readings were taken in triplicates.

Validation of the adopted HPLC method
RP-HPLC method for the identification and quantification of quercetin in chloroform extract of *Guiera senegalensis* developed by Awwalu *et al.*[17] was adopted. It was then validated in terms of intra-day and inter-day precisions.

Intra-day precision
This was determined by injecting *G. senegalensis* chloroform leaf extract, six times, at an hour interval within day and was expressed as relative standard deviation (RSD) of the relative retention time (RRT) and relative peak area (RPA).

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Inter-day precision

Inter-day precision was determined by analyzing, *G. senegalensis* chloroform leaf extract, for three consecutive days and was expressed as relative standard deviation (RSD) of the relative retention time (RRT) and relative peak area (RPA). The most prominent peak (Quercetin) in the chromatogram was chosen as the reference peak.

HPLC fingerprint analysis

Thirteen common peaks found in the chromatograms were used in the fingerprint analysis using Chemometric techniques. The most prominent peak in the chromatogram was found to be quercetin and was therefore' used as the reference peak. Degrees of similarity and difference were determined using the relationships:

Degree of similarity of a characteristic fingerprint peak = $\frac{A_I^T}{A_I^F}$ x 100%

Total degree of similarity of the characteristic fingerprint = $\frac{\sum\{A_I^T|A_I^F\}}{n}$

Degree of difference of a characteristic peak = $\frac{|A_I^F-A_I^T|}{A_I^F}$

Total degree of difference of the characteristic fingerprint =

$$\frac{\sum\langle|A_I^F-A_I^T||A_I^F\rangle}{n}$$

Where;

A_I^T = characteristic fingerprint peak area in the chromatogram of the test sample

A_I^F = characteristic fingerprint peak area in the standard fingerprint

n = total number of the characteristic fingerprint peaks, and

Σ = summation symbol

Statistical analysis

Results were analysed using IBM SPSS statistics 20 and were expressed as mean ± SD, relative standard deviation (RSD) of the relative retention time (RRT) and relative peak area (RPA) as the case may be.

Results and Discussion

The adopted method was found to resolve the various phytochemicals in *G. senegalensis* leaf without an overlap (Figure 1). It was also found to be repeatable and precise as indicated by their percents RSD of RRT and RPA of less than 3 % (Table 2). Therefore, this method is suitable for fingerprint analysis of *G. senegalensis*. The phytochemicals contained in the samples were found to be the same as evident by their superimposed chromatograms (Figure 2).

Thirteen common peaks were obtained in the chromatograms within a runtime seven minutes (Figure 2) under isocratic mode of elution. Therefore, this method could be considered very rapid and easy to use. Feng and his co-workers[18] obtained 16 common peaks for the fingerprint analysis of *Zingiber officinale* within a runtime of 110 minute using a gradient elution mode. Ten (10) common peaks were reported for *Potentilla fruticosa* within a runtime of 40 minutes under gradient elution mode.[19]

It was observed that quercetin (reference peak) eluted at 2.2 minutes (Figure 1) while in the work conducted by Liu and Co.[19] Quercetin (reference peak) eluted at 11.68 minutes. These observed differences in retention time could be due to difference in complexity of the herbs, column and mobile phase among other factors.

The degree of difference between the various samples were found to be within the range of 0.002-0.014 (Table 1) indicating slight difference in the phytochemical content between the samples. The observed slight difference was further proven by high values of the degree of similarity which were found to be close to the "one" (Table 1). Also, the low % RSD of RRT (Table 2) revealed excellent similarity between the four samples. Liu and his co-workers[19] reported high of degree similarity between two samples out of the eight samples they analyzed.

The % RSD of RPA values were within the range of 17-120 % (Table 3). These larger % RSD of RPA values indicated variation in concentrations of various phytochemicals present in *G. senegalensis* samples. These variations could be due to several factors such as temperature of the environment, soil type and age of the shrub.[20,21,22]

Table 1: Degree of Difference and Similarity of the Four Chromatograms

S/No.	Sample	Degree of Difference	Degree of Similarity
1	Ac	0.000	1.000
2	Cc	0.006	1.000
3	Dc	0.014	0.995
4	Fe	0.002	1.000

Ac = Kaduna (April), Cc = Jigawa (April), Dc = Kaduna (January) and Fe = Jigawa (January)

Table 2: HPLC Fingerprint Validation

Peak No.	Repeatability (% RSD) RRT	RPA	Precision (% RSD) RRT	RPA
1	0.09	2.55	0.98	2.75
2	0.04	1.77	0.63	1.97
3	-----	----	----	----
4	0.01	0.99	0.07	1.17
5	0.01	2.50	0.19	1.18
6	0.05	1.38	0.29	1.98
7	0.35	2.03	0.35	2.45
8	0.01	1.95	0.48	2.88
9	0.02	1.18	0.28	2.55
10	0.01	1.36	0.77	1.67
11	0.06	1.12	0.47	1.62
12	0.02	1.48	0.16	1.64
13	0.05	1.59	0.44	1.71

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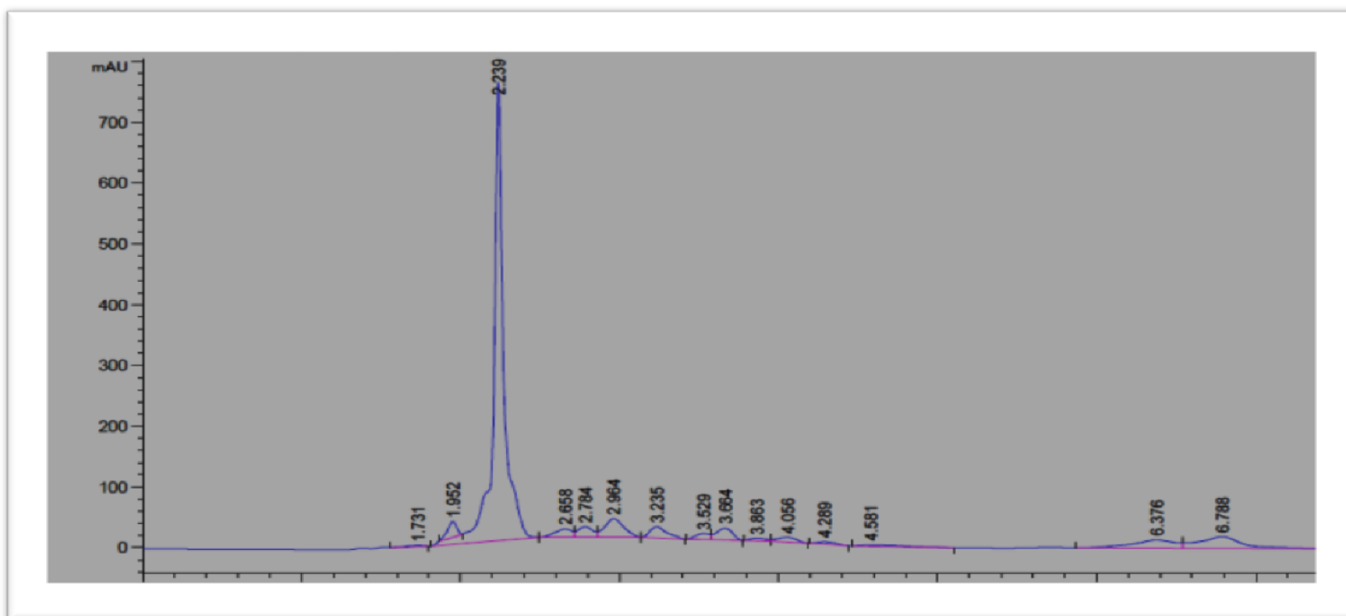

Figure 1: HPLC Chromatogram of *G. senegalensis* Leaf Chloroform Extract

Figure 2: Superimposed Chromatograms of the Samples of *G. senegalensis* Chloroform Leaf Extract

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Table 3: RRT of Common Peaks in the Four Chromatograms

Peak No.	Ac	Cc	Dc	Fe	Mean	SD	% RSD
1	0.612	0.614	0.612	0.614	0.613	0.001155	0.18836877
2	0.699	0.677	0.679	0.69	0.69475	0.019397	1.49258779
3	1	1	1	1	1	0	0
4	1.188	1.188	1.155	1.188	1.22475	0.0735	1.3986014
5	1.446	1.448	1.438	1.448	1.445	0.004761	0.32947767
6	1.576	1.578	1.576	1.583	1.57825	0.003304	0.2093482
7	1.633	1.641	1.638	1.641	1.63825	0.003775	0.23042376
8	1.723	1.731	1.727	1.731	1.728	0.00383	0.22162665
9	1.813	1.816	1.808	1.816	1.81325	0.003775	0.20818515
10	1.915	1.919	1.915	1.919	1.917	0.002309	0.12046954
11	2.045	2.053	2.045	2.049	2.048	0.00383	0.18699748
12	2.848	2.892	2.875	2.879	2.8735	0.018484	0.64326527
13	3.031	3.054	3.036	3.049	3.0425	0.010786	0.35450429

Table 4: RPA of Common Peaks in the Four Chromatograms

Peak No.	Ac	Cc	Dc	Fe	Mean	SD	% RSD
1	0.004	0.002	0.002	0.001	0.00225	0.001258	55.9246995
2	0.031	0.002	0.003	0.009	0.01125	0.013525	120.219278
3	1	1	1	1	1	0	0
4	0.029	0.017	0.162	0.05	0.0645	0.066415	102.96955
5	0.041	0.028	0.031	0.03	0.0325	0.005802	17.8532258
6	0.017	0.01	0.008	0.009	0.011	0.004082	37.113481
7	0.04	0.025	0.02	0.021	0.0265	0.009256	34.9269016
8	0.01	0.007	0.006	0.006	0.00725	0.001893	26.1099234
9	0.02	0.013	0.011	0.001	0.01125	0.007848	69.7555992
10	0.009	0.006	0.004	0.005	0.006	0.00216	36.004115
11	0.013	0.008	0.006	0.007	0.0085	0.003109	36.5779571
12	0.077	0.135	0.195	0.11	0.12925	0.049856	38.5730173
13	0.107	0.068	0.093	0.083	0.08775	0.016439	18.7342248

Conclusion

The fingerprint analysis revealed similarity in composition of the phytochemicals with differences in their concentrations. Also, the adopted method was found to be simple, rapid and reproducibility for the quality control of *G. senegalensis* leaf extract.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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