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SHORT COMMUNICATION

Seasonal Chemical Variation and Antidiabetic Activity of Major Compounds in *Artemisia afra* Infusions

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Abstract

Artemisia afra Jacq. ex Willd., Asteraceae, is a very popular herbal medicine in Southern Africa and is mainly used in the form of a tea infusion for the treatment of a wide variety of ailments, including diabetes. In this study, the phytochemical variation of four individual *A. afra* plants, collected monthly for a 1-year period, was determined. Eleven phytochemical compounds present in the infusions were identified and was used to illustrate the seasonal chemical variability. These compounds were also tested for their *in vitro* antidiabetic activity using the α-glucosidase inhibition bioassay. The results indicated that considerable phytochemical variation existed over a 1-year period within each plant but also between the four plants tested. The main bioactive compounds, namely, 1,5-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid, showed better α-glucosidase inhibition activity than the positive control, acarbose. Furthermore, these compounds appeared to show an interchangeable transition trend with its monomer, chlorogenic acid, and caffeic acid with the former seemingly increasing during summer and the latter two during the winter months. This study highlights the importance of quality control and standardisation of popular herbal remedies such as *A. afra*.

Keywords Chemical variation · Chlorogenic acids · Diabetes · α-Glucosidase · Phytochemical composition · Quality control

Introduction

Artemisia afra Jacq. ex Willd., Asteraceae, is a well-known herbal remedy in Sub-Saharan Africa. It is used to treat a wide variety of medical conditions mainly in the form of consuming an herbal tea. Even though it is most commonly used to treat flu-like conditions, it is also used for treating chronic conditions, such as diabetes. Upon review of available scientific literature, predominantly low-level evidence has been published on the activity of *A. afra* extracts and compounds used for the treatment of a wide range of ailments (Liu et al. 2009; Du Toit and Van der Kooy 2019).

The hypoglycaemic activity of *A. afra* has been reported by Afolayan and Summonu (2011) who conducted a preclinical *in vivo* analysis of *A. afra* by orally administering an aqueous extract to diabetic rats. In a subsequent study, different dosages of an *A. afra* extract were tested in Wistar rats which revealed marked hypoglycaemic activity; however, some toxicity was observed at higher dosages (Afolayan and Sunmonu 2013).

Artemisia afra was also studied for *in vitro* enzyme inhibition activity using the α-amylase and α-glucosidase bioassays. At a concentration of 0.2 mg/ml, the extract yielded an α-glucosidase inhibition of 47.2% and α-amylase inhibition of 74% compared to the positive control (acarbose) which produced 80.6% and 73.4% inhibition, respectively. This indicated that this plant has some potential as an antidiabetic agent through its enzyme inhibition activity. Issa and Bule (2015) conducted *in vivo* experiments in diabetic Swiss albino mice and tested both methanolic and aqueous extracts of *A. afra* which drastically reduced the blood glucose levels in the mice compared to that of the control group. The LD_{50} of the aqueous extract was also found to be relatively high (9 833.4 mg/kg).

The use of herbal medicines is however widely critiqued for a number of reasons, which include chemical variation

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caused by external factors. The phytochemical composition of plants can and do vary due to the plant's response to its ever-changing micro- and macro-environment. This chemical variation can therefore naturally lead to variable levels of bioactivity, which is of concern, especially when herbs are used for the treatment of chronic conditions such as diabetes. Various guidelines have been published to implement quality control measures in herbal medicines to overcome this problem; however, the effectiveness of these measures and the true extent and impact of the lack thereof are yet to be fully understood (WHO 2011; Bensoussan et al. 2015).

Plants are chemically very complex, containing many different phytochemical compounds, of which some are biologically active, and many may not be active at all. Taljaard et al. (2022) and Van Loggenberg et al. (2022) recently identified some of the phytochemical compounds present in *A. afra* infusions but did not test their antidiabetic bioactivity. This study aimed to further elucidate phytochemical compounds present in *A. afra* infusions, to follow the chemical variation of these compounds over a 1-year period and to test the major compounds with antidiabetic activity by means of α-glucosidase inhibition assay.

Materials and Methods

Ultrapure water from a Rephile Ultrapure water system (Boston, MA, USA) was used to prepare the infusions, and as solvent for the analysis. Acetonitrile and formic acid used for analysis were obtained from Sigma-Aldrich (Johannesburg, RSA). The reference standards, namely caffeic acid, 4-chlorogenic acid (4-CGA), chlorogenic acid (CGA), 1,5-dicaffeoylquinic acid (1,5-DCQA), 3,5-DCQA, 4,5-DCQA, 3,4-DCQA, ferulic acid, luteolin, neo-chlorogenic acid (neo-CGA), quercetin, scopolin, and scopoletin that were used for the comparative analysis, were purchased from Chengdu Alfa Biotechnology (Chengdu, China). All reference standards had a purity grade of > 98%.

The four *A. afra* plants that were investigated in this study were located in the Botanical Garden of North-West University (NWU), Potchefstroom, South Africa (GPS coordinates, – 26.6823° S, 27.0950° E). Three of the plants were growing in a maintained section of the garden, whilst the fourth plant grew in an unmaintained (wild) section of the garden. Moreover, plant 1 was growing in full shade whilst plants 2–4 were growing in a semi-sun environment. Samples of approximately 10 cm of the top part of a twig from each of the four plants were collected monthly to yield 12 batches per plant over a 1-year period. After collection, the material was immediately placed in an oven at 40 °C for 6 days, whereafter the dried leaves were stripped from the twigs and stored until analysis. Herbarium specimens have been deposited at the A.P. Goossens herbarium at NWU for positive identification (specimen numbers: PUC0015502, PUC0015503, PUC0015504, PUC0015505).

Table 1 provides the date, time of day, and climatic conditions on each day of collection. More exact and expanded climatic data for collection days can be assessed from a weather archive (World Weather Online 2023).

Infusions were prepared by adding exactly 1 g of plant material to 5 ml boiling ultrapure water, which were left to infuse for 10 min whilst covered. The infusions were then vortexed for 30 s to ensure proper mixing. Of each infusion, 1 ml was filtered through a 0.45-µm syringe filter into UPLC vials for analysis.

Standard solutions of the thirteen purchased reference standard compounds (caffeic acid, 4-CGA, CGA, 1,5-DCQA, 3,5-DCQA, 4,5-DCQA, 3,4-DCQA, ferulic acid, luteolin,

Table 1 Weather data in Potchefstroom for 2021 during plant collection

Sample month	Date	Time	Temp during collection (°C)	Min. temp preceding night (°C)	Average monthly temp (°C)	Average monthly rainfall (mm)	Weather conditions
1	04/01/21	07:30	25	17	24	303.7	Moderate to heavy rain showers
2	04/02/21	07:50	18	16	23	232.6	Cloudy with light rain showers
3	05/03/21	08:10	25	21	23	63.0	Partly cloudy
4	06/04/21	08:30	23	22	23	38.4	Cloudy with isolated showers
5	04/05/21	08:20	13	16	17	1.3	Partly cloudy
6	04/06/21	08:30	9	8	15	0.6	Partly cloudy
7	07/07/21	08:30	10	7	13	0.0	Partly cloudy
8	11/08/21	08:45	10	5	18	1.2	Partly cloudy
9	10/09/21	08:15	19	14	24	2.2	Partly cloudy
10	04/10/21	08:10	20	14	24	22.2	Partly cloudy
11	04/11/21	07:30	27	19	16	32.3	Cloudy with scattered thundershowers
12	06/12/21	08:15	26	16	23	55.6	Sunny

neo-CGA, quercetin, scopolin, and scopoletin) were prepared by accurately weighing 1 mg of each compound into individual 2-ml amber UPLC glass vials. The compounds were then dissolved with 1 ml of methanol to yield a 1000 µg/ml concentration. A volume of 100 µl of each reference standard solution was mixed together in another vial to yield a stock mixed solution with a final concentration of 76 µg/ml of each marker.

The samples were analysed on a Shimadzu i-Nexera ultra-high performance liquid chromatography (UPLC) system equipped with a quaternary pump, an auto sampler, and a photodiode array detector. The system was fitted with an Agilent Poroshell 120 EC-C18, 3×150 mm, 2.7-µm column with the column oven set to 35 °C. The solvent system consisted of water + 0.1% formic acid (A) and acetonitrile (ACN) + 0.1% formic acid (B) and a step-wise gradient system was employed: 0 min, 10% B; 5 min, 10% B; 6 min, 20% B; 10 min, 20% B; 11 min, 85% B; 16 min, 85% B; 16.10 min, 100% B; 17 min, 100% B, 17.10 min, 10% B, 20 min 10% B. UV detection was carried out at 254 nm and UV spectra were collected between 190 and 800 nm. The flow rate was 0.49 ml/min and 10 µl of each sample was injected. After every 12 samples, a blank injection was conducted in order to test for and prevent any carry over between samples.

The method used to determine the enzyme inhibition was adapted from Kazeem et al. (2013). α-Glucosidase from *Saccharomyces cerevisiae* was used to determine the α-glucosidase inhibition of selected phytocompounds present in the *A. afra* infusions. A *p*-nitrophenyl glucopyranoside (pNPG) solution was prepared in 20 mM phosphate buffer (pH 6.9). A volume of 100 µl α-glucosidase (1 U/ml) solution was preincubated with 50 µl of the reference standards (1 mg/ml) for 10 min at 37 °C in a transparent flat-bottom 96-well plate (Sarstedt AG, Sevelen, Switzerland). A volume of 50 µl of 3 mM pNPG solution in phosphate buffer (pH 6.9) was added to the α-glucosidase/reference standard solution as the substrate to start the reaction. The reaction mixture was incubated at 37 °C for 20 min. The activity was determined by measuring the yellow-coloured paranitrophenol released from the pNPG at 405 nm using a Spectramax™ Paradigm plate reader. All experiments were conducted in triplicate and averages were obtained. Distilled water and acarbose were used in the place of the reference standards as negative and positive controls, respectively. Once the absorbance was measured, the % inhibition of each sample was calculated using the following formula:

$$\% \text{ Inhibition} = \left(\frac{\text{AbsNegative Control} - \text{AbsExtracts}}{\text{AbsNegative Control}} \right) \times 100$$

Results and Discussion

The overlayed chromatograms of the 12 samples collected monthly from each plant are presented in the supplementary data (Fig. 1S). It is clear from the chromatograms that a large degree of chemical variation was present not only between the individual plants, but also in samples collected over the course of the year. In order to better visualise these differences, four seasonal samples (January, April, July, and October) were selected from plant 1 (growing in full shade) and plant 2 (semi-sun). The chromatograms from the eight selected samples as well as the reference standard mixture solution is given in Fig. 1. The most striking difference was the class of compounds eluting between 16.5 and 18 min in samples prepared from plant 1. These compounds were completely absent in plants 2–4 with the main difference being that plant 1 grew in a shaded environment, whilst the other samples received direct sunlight (Fig. 1S). The choice of 254 nm was made after investigating different wavelengths between 220 and 330 nm. The 254-nm chromatogram was found to be the most 'populated' and was therefore selected. An overlayed chromatogram extracted at 254 nm and 330 nm can be found in the supplementary material (Fig. 2S).

Moreover, not all of the selected reference compounds were present in high concentrations with luteolin and quercetin being undetectable using UPLC. A number of unknown major compounds, which are represented by the peaks eluting at 9 min in plant 1, and compounds eluting at 10 and 11 min in plant 2, remain unidentified.

The seasonal comparative analysis confirmed the large chemical variation within the same plant and between the plants over the course of a year. The flavonoids quercetin and luteolin were detected but were present at very low levels. These flavonoids, which are considered to play a significant role in the general bioactivity of *Artemisia* spp., are therefore either present in low concentrations in the plant or are not efficiently extracted with water due to their non-polar nature.

A recent study where 6 marker molecules were analysed indicated an interchangeable nature in the seasonal quantity between CGA and 4,5-DCQA (Olivier et al. 2023). Our study looked at caffeic acid, 3 CGA derivatives, and 4 DCQAs and seems to confirm this observation. During winter months, the DCQAs were at their lowest levels present in the plant, whilst CGA and caffeic acid were at their highest concentrations. In plant 1, which was grown in full shade, this chemical interchangeability was less pronounced. This trend could possibly be ascribed to the difference in molar attenuation coefficients between CGA (18.3 ϵ) and DCQA (34.0 ϵ) at 329 and 330 nm, respectively. This implies that the biosynthesis of DCQA

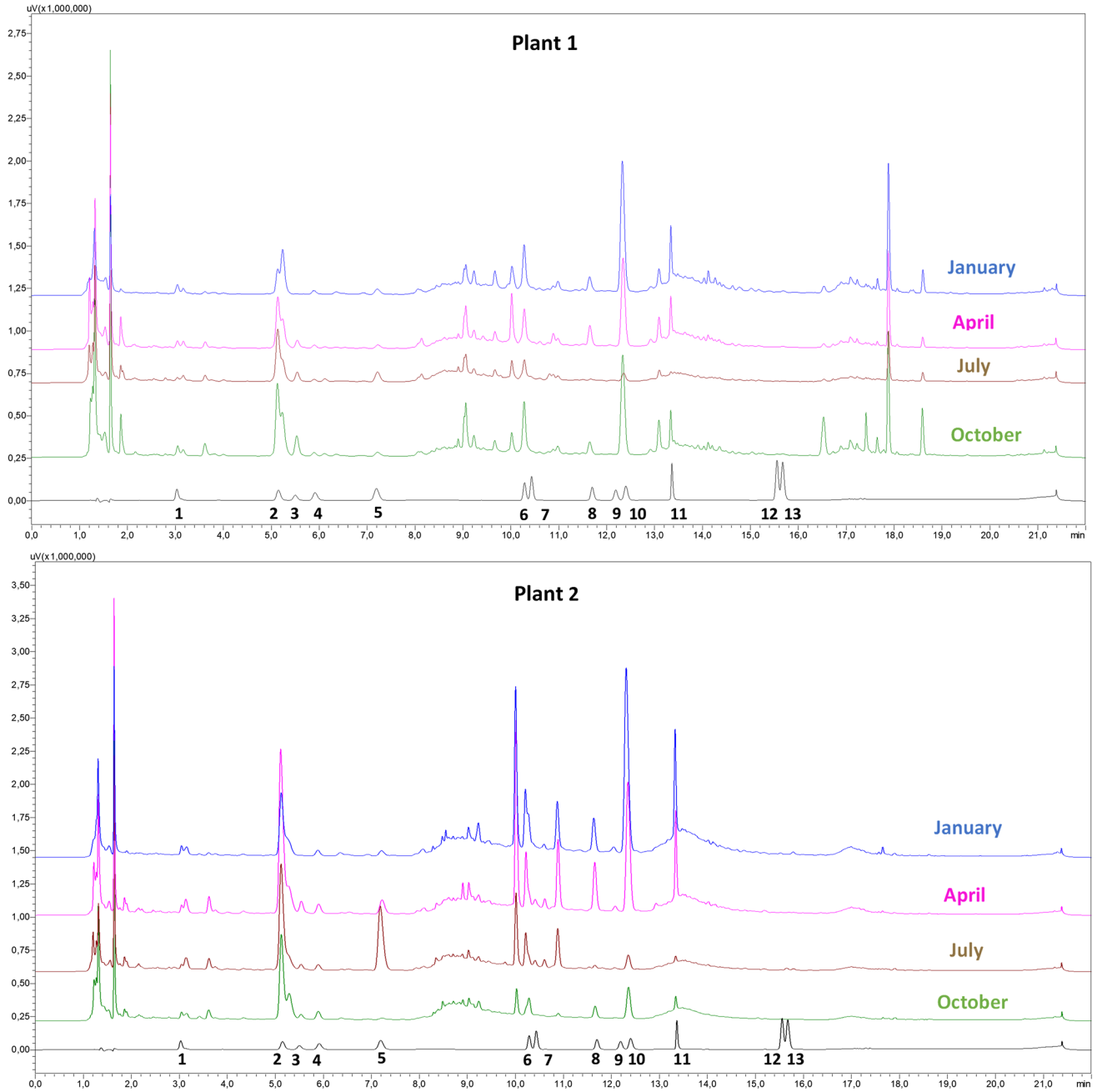

Fig. 1 UPLC chromatograms of the selected *Artemisia afra* samples as well as the 13 reference standards: (**1**) neochlorogenic acid, (**2**) chlorogenic acid, (**3**) scopolin, (**4**) 4-CGA, (**5**) caffeic acid, (**6**) scopoletin, (**7**) ferulic acid, (**8**) 3,4-DCQA, (**9**) 1,5-DCQA, (**10**) 3,5-DCQA, (**11**) 4,5-DCQA, (**12**) luteolin, (**13**) quercetin

is likely increased during summer months to protect the plant against higher levels of UV radiation (Kaeswurm et al. 2021). Lazzari Almeidaa et al. (2017) reported a similar trend occurring in *Mikania laevigata* Schultz and *M. glomerata* Sprengel, Asteraceae.

Most of the other phytochemical compounds in the *A. afra* plants showed an 'expected' gradual decrease in concentration during winter and an increase in concentration during summer.

Table 2 shows the enzyme inhibition results for the 13 reference compounds, eleven of which was identified in the *A. afra* samples. Interestingly, two of the four DCQA derivatives (1,5-DCQA and 3,5-DCQA) showed enzyme inhibition activities above 80%, compared to the positive control,

Table 2 *In vitro* bioassay results obtained by the 13 reference compounds

Sample	% Inhibition ± SD*
Caffeic acid	4.05 ± 0.02
4-CGA	NI
CGA	31.05 ± 0.06
1,5-DCQA	88.96 ± 0.02
3,5-DCQA	84.69 ± 0.03
4,5-DCQA	25.55 ± 0.12
3,4-DCQA	43.96 ± 0.58
Ferulic acid	11.74 ± 0.01
Luteolin	26.09 ± 0.04
Neo-CGA	18.52 ± 0.05
Quercetin	35.68 ± 0.02
Scopolin	2.43 ± 0.26
Scopoletin	NI
Acarbose**	47.54 ± 0.07

*All experiments were performed in triplicate ($n = 3$) and the results given as average ± standard deviation (SD)

**Positive control reconstituted in buffer

NI no inhibition

acarbose, which inhibited α-glucosidase only at 47.5% at the same concentration of 1 mg/ml. Furthermore, CGA inhibited the enzyme only at 31.05%. Etemadi-Tajbakhsh et al. (2020) reported a similar trend where 1,5-DCQA showed higher enzyme inhibition than CGA. El-Askary et al. (2022) tested 3,5-DCQA, 3,4-DCQA, and 4,5-DCQA for their α-glucosidase inhibition and reported that 3,5-DCQA had the most activity followed by 3,4-DCQA with 4,5-DCQA being the least active. The results obtained in this study confirmed this with 3,5-DCQA showing better activity than the positive control (acarbose) whereas 1,5-DCQA was found to be the most active. El-Askary et al. (2022) reported very weak toxicity for the above-mentioned compounds. The other phytochemical compounds tested for enzyme inhibition showed moderate (quercetin) to low inhibition with 4-CGA and scopoletin showing no inhibition. The crude infusions of *A. afra* were also tested but due to the intense color of the infusions, no reliable result could be obtained.

The results from this study confirmed the well-known phenomenon of phytochemical variation in plants based on the season as was also published by Sotenjwa et al. 2020. In this study, it was indicated that this variation also occurred between plants growing in a very similar environment (soil type, climatic conditions), but with different sunlight exposures. The main *in vitro* bioactive compounds were found to be 1,5-DCQA and 3,5-DCQA which showed better α-glucosidase inhibition activity than the positive control acarbose. *In vitro* activity can, however, not be directly correlated with treating DM but should only be viewed as indicative of possible *in vivo* activity. This study does however highlight some of the (expected) difficulties in ensuring consistent (chemical) quality and the need for standardisation of popular herbal medicines such as *A. afra*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43450-024-00527-6.

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Declarations

Ethics Approval Not applicable.

Competing Interests The authors declare no competing interests.

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