

¹H NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY REVEALS SECONDARY METABOLIC VARIATIONS OF SPECIAL TEA (*MONSONIA BURKEANA* PLANCH. EX HARV) POPULATIONS FROM THREE SELECTED LOCATIONS IN SOUTH AFRICA

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Abstract. The present study was conducted to assess the secondary metabolite profile differences of *Monsonia burkeana* populations naturally grown at Chuenespoort, Zebediela and Rietondale in South Africa. Three field surveys were conducted on 14 January 2015, 11 January 2016 and 4 May 2016 respectively in Chuenespoort, Zebediela and Rietondale and secondary metabolite profiles of the plants were determined using ¹H nuclear magnetic resonance (NMR) spectroscopy coupled with principal component analysis (PCA). Leaf samples from three plants per locality in the first survey and fourteen plants per site in subsequent samplings were included in the NMR analysis. Based on ¹H NMR spectroscopy and PCA of secondary metabolite profile data no major differences were detected between the populations except for the first survey where the Zebediela and the Chuenespoort populations were more closely related to each other than the Rietondale population. This study has for the first time determined differences between three plant populations of special tea with regards to secondary metabolite profiles.

Keywords: *plant population analysis, Monsonia burkeana, secondary metabolites, nuclear magnetic resonance, principal component analysis*

Introduction

Special tea (*Monsonia burkeana* Planch. ex Harv) (*Fig. 1*) is a prominent medicinal plant which is used to cure minor ailments (Venter, 1979) and it is native to Southern African countries such as Botswana, Lesotho, Mozambique, Namibia, Swaziland and South Africa (Wells et al., 1986; Gibbs Russel et al., 1987). This tea plant was recently reported in Madagascar (Schatz et al., 2011). Over and above indigenous knowledge various studies have uncovered the potential health benefits of special tea, among them, anti-diabetic and anticancer properties (Ngoepe et al., 2018; Mathivha, et al., 2019). Various biological and physical characteristics determine plant growth, plant traits and chemical composition, and therefore plant populations of various habitats are likely to differ in average plant height, average stem diameter, average number of leaves per plant, average dry biomass, leaf fat content, leaf protein content and secondary metabolite profiles (Khokhar and Magnusdottir, 2002; Clayton et al., 2006; Ohno et al., 2011; Von Staszewski et al., 2011; Sabhapondit et al., 2012; Lee et al., 2014a). For a wild tea plant which is harvested and traded it is important to determine if different populations are affected by their habitat characteristics so that productivity can be predicted and the harvested product quality can be standardized. Three populations of *M. burkeana* were selected for the assessment.



Figure 1. Special tea (*Monsonia burkeana* Planch. ex Harv) plant growing in Rietondale, Gauteng Province, South Africa

Nuclear magnetic resonance (NMR) spectroscopy coupled with principal component analysis (PCA) have been employed to evaluate the quality of foods and drugs and to evaluate drug toxicity (Khokhar and Magnusdottir, 2002; Ohno et al., 2011; Lee et al., 2014a; Martínez-Richa et al., 2003; Morita et al., 2004; Morita et al., 2008; Jiang et al., 2015). The use of this approach has been extended to plant population studies to assess differences as well as gauge various traits like plant population survival. Thus, in this study whose aim was to assess differences between three plant populations of special tea naturally grown in Chuenespoort, Zebediela and Rietondale and secondary metabolites profile differences of special tea populations were revealed using NMR coupled with PCA.

Materials and methods

Study area

Three sites, namely, Zebediela (Long: 24° 11' 6" S and Lat: 29° 27' 42" E), Chuenespoort (Long: 24° 10' 56" S and Lat: 29° 27' 29" E) and Rietondale (Long: 25° 43' 43" S and Lat: 28° 14' 19" E) were selected for the study. Zebediela and Chuenespoort are in the Limpopo province of South Africa about 20 km apart and Rietondale is in the Gauteng province about 300 km from the other two sites (Figs. 2 and 3).

These sites are isolated and natural except for Chuenespoort which was a maize field. However, no apparent disturbance took place at these sites, including Chuenespoort, during the survey period. Three field surveys were conducted on 14 January 2015, 11 January 2016 and 4 May 2016 as shown in *Table 1*. The climate data was included to show one of the aspects that vary between the sites but no aspect of climate is part of the investigation.



Figure 2. A modified Google map of the north-eastern South Africa showing the study sites, Zebediela, Chuenespoort and Rietondale where the leaf samples of *Monsonia burkeana* were collected for secondary metabolite profiling. The city of Johannesburg is circled in black

Table 1. Average daily rainfall and maximum temperatures in the 90 days before sampling in Zebediela (Z), Chuenespoort (C) and Rietondale (R). Notice that the Rietondale site received more rain and was warmer than the other two sites

Survey	Average daily rainfall (mm) 90 days before sampling	Average maximum temperature (°C) 90 days before sampling
Z ₁ 14 January 2015	70.0	26.9
C ₁ 14 January 2015	70.0	26.9
R ₁ 14 January 2015	130.0	28.9
Z ₂ 11 January 2016	32.2	29.0
C ₂ 11 January 2016	32.4	29.0
R ₂ 11 January 2016	62.0	31.3
Z ₃ 04 May 2016	50.0	27.0
C ₃ 04 May 2016	50.0	27.0
R ₃ 04 May 2016	102.0	28.7

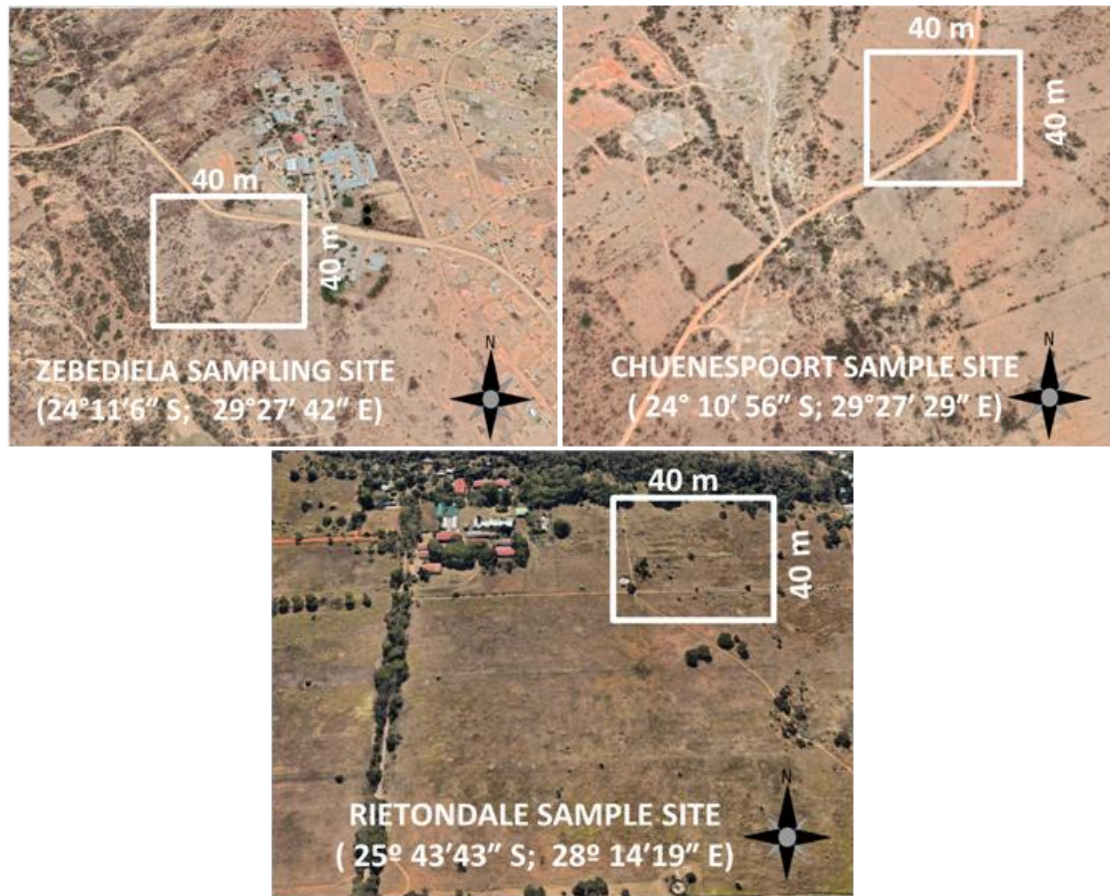


Figure 3. Study sites Zebediela, Chuenespoort and Rietondale showing the demarcation of unitary quadrats in which leaf samples of *Monsonia burkeana* were collected for secondary metabolite profiling

Secondary metabolite profiles of the plants were determined, three plants per locality in the first survey, fourteen plants per site in subsequent samplings. Secondary metabolite profiles were determined by ¹H NMR using a 600 MHz NMR Varian spectrometer to obtain magnetic spectra of the samples. Both phase and baseline corrections of the spectra were done using the ACD/NMR Processor. Only the most visibly discriminant range (assessed from spectra) was included in the bucketing for PCA. After processing the spectra, the intensity values represented by the spectra were exported as ASCII files and these intensity values were displayed on MS Excel to form a matrix which was used for PCA. PCA reduces dimensions of a dataset. It works by using an orthogonal transformation to convert a set of possible correlated variables into what is referred to as principal components. The principal components are designed in such a way as to explain as much of the possible variability. The first principal component has the largest possible variance and incrementally from the first principal component to successive principal components the possible variance decreases. For the dataset obtained in this study, the PCA was done using Multibase. Datapoints which represented NMR intensity values of the secondary metabolites extracted from the leaves of the plant samples from each of the localities were spread along PC 1 and PC 2. On the PCA scatterplot ellipses were drawn to show the discrimination between the localities.

Results

Secondary metabolite profiles which were determined by ^1H NMR spectroscopy, slightly varied or did not vary at all between the populations of Zebediela, Chuenespoort and Rietondale. Zebediela and the Chuenespoort populations were more closely related to each other than the Rietondale population in the first survey as shown by the PCA plot (Fig. 4) and the spectra shown in Figure 5.

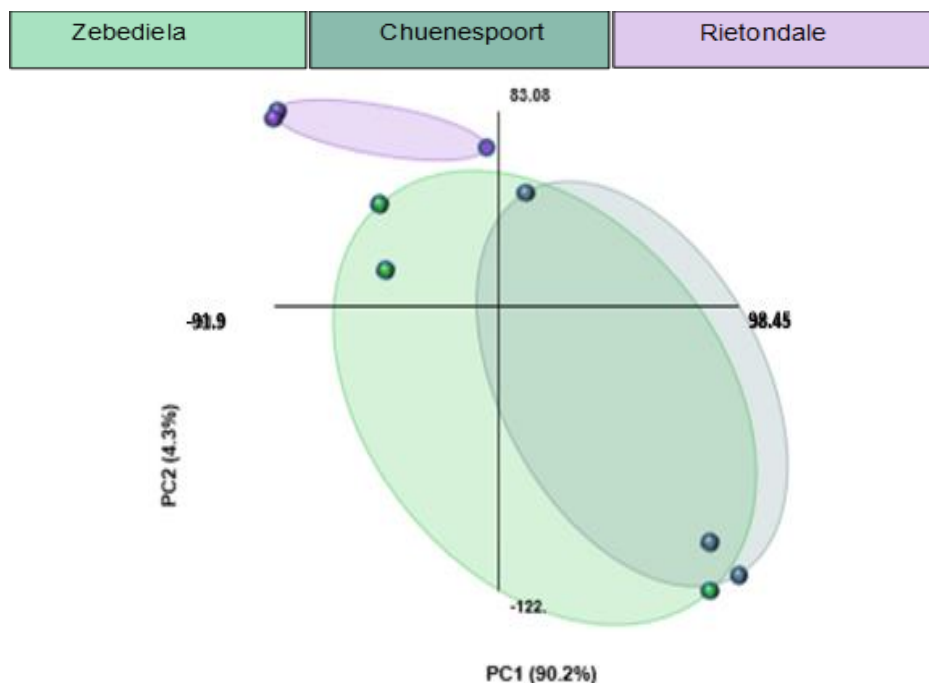


Figure 4. Principal component analysis plot of ^1H NMR intensity values of secondary metabolites of *Monsonia burkeana* leaf samples from Zebediela, Rietondale and Chuenespoort. The sampling dates was 14 January 2015. The PCA plots show that the Rietondale site is slightly different from the other two sites which are similar

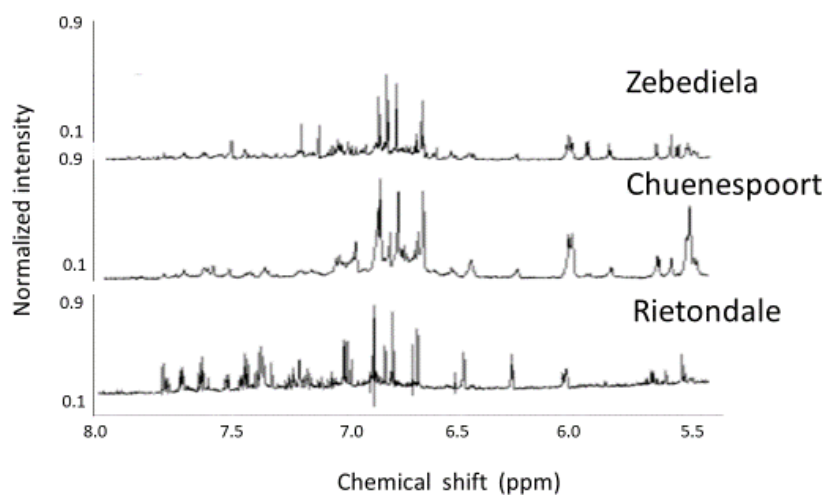


Figure 5. ^1H NMR spectra of *Monsonia burkeana* leaf secondary metabolites in the 8.0–5.5 ppm range. Sampling was done in 14 January 2015. Notice that the spectrum of the Zebediela locality and Chuenespoort's are visible different from the Rietondale spectrum

In subsequent surveys, no difference in secondary metabolite profiles were detected in the different plant populations. The differences in the ¹H NMR spectra of special tea leaves from three plant populations shown in *Figure 5* was clearly as a result of differences in the 5.5–8.0 ppm range which was selected from the original shift scale which was from 0 ppm to 14 ppm. Within this discriminant region, it was obvious that the segregation was further determined by the region between 6.5 ppm and 7.8 ppm. The regions of sharp differences between the Rietondale spectra and either of the spectra, Zebediela and Chuenespoort must have caused the separation of the Zebediela ellipse from the other ellipses in the PCA plot in *Figure 4*.

Discussion

The current study revealed slight differences in the secondary metabolite profiles of three areas in one of three samplings conducted on 14 January 2015, 11 January 2016 and 4 May 2016. Studying natural plant populations is always challenging because of the inability to determine all interacting factors. The authors of this manuscript intended just to assess differences in the secondary metabolomic profiles of *M. burkeana* populations in Zebediela, Chuenespoort and Rietondale without investigating all underlying biophysical factors. However, it was found necessary to obtain rainfall and temperature data since rainfall and temperature are assumed to be major determinants of the growth and survival of the plant. The Rietondale site which is located about 300 km south of the other two sites received a little more rain and was also slightly warmer than the other sites. Despite these observable differences it was not possible to assume that these climatic differences were the main causes of the marked secondary metabolite profile differences observed in the samples collected on the 14 January 2015. A more comprehensive study would be necessary to assess this aspect. The scope of this work was only limited to assess if there are any differences in the secondary metabolomic fingerprint. However, it was unsurprising to find some differences in the secondary metabolite profiles of the study populations given the differences between plant populations caused by factors such as rainfall, temperature, soil type and altitude. The difference in secondary metabolites may be attributed to variation in rainfall received during the sampling periods. It was noted that in the first survey of the current study high rainfall was recorded as compared to the subsequent two surveys where low rainfall was received. A study conducted by Wedeking et al. (2018) indicated that drought stress triggers various physiological and biochemical responses in plants. The exposure of plants to various stress conditions results in modulations in metabolite contents and altered metabolic interactions, caused by alterations in environmental parameters, such as light, temperature and water (Mittler, 2006).

Secondary metabolism of plants may change considerably due to the influence of several biotic and abiotic stress signals (Pavarini et al., 2012). The finding of the current study is in agreement with Lee et al. (2014b) who found that secondary metabolites from the *Curcuma* spp. from different geographical locations using PCA was not different between the locations. However, this is in disagreement with Martin et al. (2016) who found that secondary metabolites from *Galium odoratum* showed clear differences between the plants from nature and those of controlled growth conditions as well as internal variation within the group. Lee et al. (2010) studied the effects of climatic conditions on *C. sinensis* metabolites in three different growing areas of Jeju Island, South Korea using ¹H NMR spectroscopy and found the revealed clear

discriminations of green teas from the three different growing areas. The ¹H NMR technique itself provides a solution for the identification and quantification of metabolites in plant cell, tissue, organ and extracts (Halabalaki et al., 2014; Zhou et al., 2017). Both PCA and orthogonal projections to latent structures discriminant analysis (OPLS-DA) revealed clear discriminations of green teas from the three different growing areas in terms of theanine, isoleucine, leucine, valine, alanine, threonine, glutamine, quinic acid, glucose, epicatechin (EC), epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG), and caffeine levels. The PCA revealed a separation between the Longjing type and other Chinese teas in metabolites differences. Changes in seasonal climatic conditions affect flavanol and purine alkaloid contents of two leaves and a bud of tea (Wang et al., 2011). Lengthy period of precipitation decreases EGC, EC, ECG, EGCG and their total content but increases C content, whereas increased daily average temperature decreases the levels of EGC, EC, ECG, EGCG and C in green tea. The EGCG, CG and total catechins (TC) in *C. sinensis* grown at a high altitude are higher than those grown at a low altitude (Chen et al., 2010).

Nchabeleng et al. (2012) determined the chemical composition of wild bush (*Athrixia phylicoides* DC.) tea growing at locations differing in altitude, climate and edaphic factors. There was a positive correlation ($r^2 = 0.55$) between total polyphenol content of *A. phylicoides* and altitude. Samples of *Angelica gigas* collected from different geographical regions were distinguished by Kim et al. (2011) using ¹H NMR spectroscopy and ultraperformance liquid chromatography–mass spectrometry (UPLC-MS) followed by multivariate data analyses. The study concluded that ¹H NMR and UPLC-MS-based metabolic profiling coupled with chemometric analysis can be used to discriminate the geographical origins of various herbal tea. The variations of the total and individual catechin contents of tea were evaluated among three cultivars grown in 10 locations; the dynamic changes of chlorophyll and catechin contents in two cultivars during young leaf development were also analysed. This study highlights how metabolomics coupled with multivariate statistical analysis can illuminate the metabolic characteristics of *M. burkeana*. This application can be extended to do special tea quality studies.

Conclusion

In conclusion, based on ¹H NMR and principal component analysis of secondary metabolite profile data no major differences were detected between the populations except only in the first survey when the Zebediela and the Chuenespoort populations were more closely related to each other than the Rietondale population. Follow-up studies must focus on an in-depth understanding of the various causes of secondary metabolite profile differences between populations in various localities in South Africa. Discriminatory secondary metabolites must be identified using various applications from NMR to chromatography coupled with mass spectrometry. Moreover, future studies should determine the growth potential of *M. burkeana* under controlled conditions, and further metabolomic profiling of the special tea growing under controlled conditions.

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