The Potential Effect of Elevated Root Zone Temperature on the Concentration of Chlorogenic, Caffeic, and Ferulic acids and the Biological Activity of Some Pigmented Solanum tuberosum L. Cultivar Extracts

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Abstract: Without a doubt, potatoes play a vital food and nutrition security role in the world as more than a billion people consume this vegetable. Furthermore, the polyphenolic constituents of pigmented potato cultivars and their associated health benefits have been reported. However, the antioxidant, anticancer, and antimycobacterial activity of pigmented cultivars are scanty. Therefore, the present study explores the phenolic acids and biological activities of cv. Salad Blue (SB) and non-pigmented control (BP1) extracts. The antiproliferative activity of S. tuberosum L. against human hepatocellular carcinoma (HepG2) was investigated, as well as the ability to inhibit Mycobacterium smegmatis. Chlorogenic acid was the most prominent phenolic acid in both treatments as well as cultivars. In the current trial, 24 °C significantly increased chlorogenic acid in cv. SB and BP1. Ethanolic extracts of all the samples showed no activity at the highest test concentration of 1000 µg/mL (ciprofloxacin MIC of 0.325 µg/mL) against M. smegmatis. The antiproliferative activity of the tuber samples against HepG2 liver cells had IC50 values ranging between 267.7 ± 36.17 µg/mL and >400 µg/mL. Since the health benefits of these cultivars are highly valued, the present study provides useful information for future oncology studies, for human nutrition, as well as for how these underutilized cultivars can be fortified to improve their health benefits.

Keywords: Solanum tuberosum; antimycobacterial; antioxidant capacity; hepatocellular carcinoma; pigmented potatoes

1. Introduction

Of the crops that feed the world, potatoes are the third most consumed after rice and wheat, with more than a billion people relying on them for food and nutrition security [1]. This is because they are an important staple crop that is well endowed with complex carbohydrates and thus very high in energy, in addition to other nutritional benefits. This crop is easy to cultivate under a diverse range of climatic conditions except those found in Antarctica [2]. Latest statistics indicate that by 2019, over 17 million hectares were under potato cultivation. Global production also increased from 334.73 to 370.43 million metric tons (MMT) between 2009 and 2019 [2]. Current data [3] further show that global leaders in potato production by MMT are the following: China (91.92) > India (50.19) > Russia (22.07) > Ukraine (20.27) > USA (19.18) > Germany (10.60) > Bangladesh (9.65) > France (8.56) > Netherlands (6.96) > Poland (6.48).
In South Africa, potatoes also play a major food and nutrition security role, with about 677.46 km² under potato cultivation in the 2016/2017 agricultural season [4]. Furthermore, during the 2016/2017 agricultural season, about 2.5 MMT of potatoes were produced at a rate of approximately 3,617,200 kg/km² [4]. According to the same statistics, the average per capita consumption of potatoes in South Africa is 30 kg.

The usefulness of the potato peel, which is regarded as a waste product, has grown [5,6]. Due to its high antioxidant and antimicrobial effectiveness [7], it is used in food preservation. The potato peel has also been reported as a pharmaceutical ingredient in wound management [8,9], and glycoalkaloids in the leaves and other parts of the plant have been reported to offer natural protection against plant pests [10,11].

In the world today, the demand for polyphenolic and vitamin rich food sources is on the rise, and pigmented potatoes, among other crops, often possess these constituents [12].

Potatoes are rich in phenolic compounds; for example, about 49% to 90% of the total phenolics in this vegetable are in fact chlorogenic acid [13,14]. In addition, as shown in some epidemiological studies, a clear correlation has been established between consuming phenolic rich diets and better health [15–18]. Nevertheless, the antioxidant activity and some health promoting aspects of plant-derived phenolic compounds cannot be overemphasized. Anthocyanins in some pigmented potato cultivars have been reported to suppress stomach cancer in mice [19], prostate cancer cells [20], colon cancer cells [21], as well as liver cancer cells [22]. Furthermore, glycoalkaloids in these cultivars have been shown to inhibit the growth of colon, liver, stomach, and lymphoma cancer cells, among others [23,24]. However, the potential role of root zone temperature (RZT) on antioxidant, anticancer, as well as antimycobacterial activity of pigmented cultivar extracts has not been reported. We previously reported root zone temperature’s role on physiological growth and polyphenolic contents in both pigmented and non-pigmented potato cultivars [25]. The results of this study showed the polyphenolic superiority of pigmented cultivars under a diverse range of root zone temperatures over the non-pigmented cultivar. Therefore, as an offshoot of this study, we tested the antiproliferative activity of the extracts of both the pigmented and non-pigmented cultivars against liver hepatocellular carcinoma (HepG2) cells and their antimycobacterial activity against M. smegmatis. In addition, chlorogenic and caffeic acid were significantly higher in SB under 24 °C in our previous study, and this informed our decision for the selected temperature and cultivar of the current study.

2. Materials and Methods
2.1. Plant Growth, Harvest, and Extraction

Potato tubers of cv. BP1 and SB were cultivated in a greenhouse, as described by [25], for 73 days to test the effect of controlled RZT (24 °C) and non-controlled RZT (ranging between 19 °C and 25 °C). Postharvest, the samples were separated into flesh and skin, and frozen at −80 °C in paper bags, after which they were freeze dried for 24 h in a VirTis genesis wizard 2.0 (UK). The samples were then powdered and sieved through a 40–60 mesh and stored at 4 °C until further use. About 200 g of the powdered and lyophilized tubers were extracted with 20 × the volume of 60% ethanol (EtOH) (Absolute; B&M Scientific) per mass (10 g > 200 mL) overnight and then ultra-sonicated for approximately 15 min at 40 °C. Samples were filtered using 0.22 µm syringe filters (25 mm) and concentrated using the Genevac miVac sample concentrator.

2.2. Phytochemical Analysis

A Dionex HPLC (Dionex Softron, Germering, Germany) was used to determine chlorogenic, caffeic, and ferulic acids in the samples. This HPLC has a Brucker ESI Q-TOF MS coupled autosampler and is equipped with a binary solvent manager. A reversed chromatography on a Thermo Fisher Scientific C18 column 5 µm, 4.6 × 150 mm (Bellefonte, PA, USA) was used to separate plant extract constituents via the use of a linear gradient of 0.1% formic acid in acetonitrile (solvent A) and water (solvent B) at 0.8 mL min⁻¹ flow rate, an electrospay voltage of +3500 V, an oven temperature of 30 °C, and a 10 µL injection
volume. The negative mode was used to acquire the MS spectra. The nebulizer gas was set at 35 psi and the dry gas to 9 L min^{-1} at 300 °C.

2.3. Antimycobacterial Activity of the Ethanolic Extract of Solanum tuberosum L.

*Mycobacterium smegmatis* (M. smegmatis) is a non-pathogenic and fast-growing species of mycobacterium. This model is most used in the physiology of mycobacteria, as it has relevance to the pathogenic species of *Mycobacterium tuberculosis*, the causative pathogen for tuberculosis. The minimum inhibitory concentration (MIC) values were determined according to [26], with slight modifications.

A stock solution of 20% DMSO (Sigma-Aldrich, Saint Louis, MO, USA) was used to dissolve all tuber ethanolic extracts in Sterile Middlebrook 7H9 media. Furthermore, using this sterile media, two-fold dilutions were made of each sample into a final assay yield volume of 200 µL. Ciprofloxacin (Sigma-Aldrich, Saint Louis, MO, USA) served as a positive drug control at a concentration range of 0.156–10 µg/mL. The solvent control (DMSO 2%) as well as the untreated bacterial control were carried out in triplicates. The plates were sealed using parafilm before incubation at 37 °C for 24 h. After incubation of 24 h, PrestoBlue (Thermofischer, South Africa) as a viability indicator was added to each well (20 µL). The minimum inhibitory concentration (MIC) values were defined as the concentration at which no color change was visible from blue to pink.

2.4. Antiproliferative Activity

The antiproliferative activity assay was carried out according to the method of [27]. In all, 100 µL of HepG2 cells with a cell density of 10,000 cells per well was seeded in 96 well plates after careful counting and left at 5% CO₂ and 37 °C overnight to incubate in order to allow for attachment. To prepare each sample, a stock solution of 2000 µg/mL was used. A final test concentration of 12.5 to 400 µg/mL in serial dilutions of the sample extracts in the cell-containing plates was made. The plates were then incubated for 72 h at 37 °C and 5% CO₂. Actinomycin D was used as the positive control (0.02 to 0.5 µg/mL) and DMSO at 2% as the solvent control. After incubation, PrestoBlue was added (20 µL) to each well, and the plates were left to incubate for a further 2–4 h. After incubation, the absorbance values were read (490 nm wavelength, including a reference wavelength of 690 nm) using a BIO-TEK Power Wave XS multi-well reader. The mean 50% inhibitory values (IC_{50}) were calculated, and statistical analysis was performed.

2.5. Statistical Analysis

Data were collected on 52 samples (13 plants per cultivar) per treatment. Statistically significant differences among treatment means were determined by two-way analysis of variance (ANOVA) at *p* < 0.05. Fisher’s least significant difference (LSD) test was used to segregate means that were significantly different using a computer software program called STATISTICA (Palo Alto, California, USA). The mean IC_{50} values (three replicates) were used to perform statistical analysis using GraphPad Prism (Version 7, San Diego, CA, USA) and two-way ANOVA. To identify significance in comparison to the control value, the Dunnett’s MCT was performed. All experiments were conducted in triplicates.

3. Results

3.1. Caffeic, Chlorogenic, and Ferulic acid Content in Solanum tuberosum L. Exposed to Higher Root Zone Temperature

The results of the present study revealed the presence of chlorogenic, caffeic, and ferulic acids in the ethanolic extracts of the potato tuber cultivars, as shown in Table 1. The chromatographic peaks in the result profiles showed some variations in the mean concentrations among the two root zone temperatures and the cultivars. Chlorogenic acid was the most prominent phenolic acid in both treatments and cultivars. Cultivar BP1 flesh and skins increased chlorogenic acid by 13% and 26%, respectively, on exposure to an RZT of 24 °C. Similarly, cv. SB flesh and skins increased by 28% and 46%, respectively, on
exposure to an RZT of 24 °C. Although the set RZT of 24 °C significantly lowered caffeic acid in cv. BP1 skins and flesh (0.29–0.03 µg/g), the same RZT significantly increased this specific phenolic acid in the cv. SB skins (0.39 µg/g) and the flesh (0.05 µg/g). Interestingly, the control temperature (no heat applied) significantly increased the concentration of caffeic acid in BP1 skins (0.367 µg/g) and decreased the concentration in the flesh (0.051 µg/g). In addition, as shown in Table 1, at 24 °C the concentration of chlorogenic acid in cv. BP1 skins was lowered to 0.530 µg/g and in the BP1 flesh to 0.531 µg/g; however, a set temperature of 24 °C had the ability to increase the concentration of chlorogenic acid in cv. SB skins (0.779 µg/g) and the flesh (0.707 µg/g). The control temperature significantly lowered the concentration of the chlorogenic acid in both cultivars. Ferulic acid was present, but in very low concentrations only in cv. SB. Using a two-way analysis of variance, a strong interaction was established between the specific cultivar and RZT on chlorogenic and caffeic acid contents in the present trial.

Table 1. The effect of root zone temperature on the phenolic acid content in S. tuberosum cv. BP1 and Salad blue.

| Phenolic Acid          | Control | 24 °C | Control | 24 °C | Control | 24 °C |
|------------------------|---------|-------|---------|-------|---------|-------|
| **Caffeic Acid (µg/g)**| BP1 Skins | 0.37 ± 0.004 aA | 0.29 ± 0.003 bB | 0.39 ± 0.006 bC | 0.53 ± 0.002 aC | 0.00 ± 0.001 |
|                        | BP1 Flesh | 0.05 ± 0.001 c | 0.03 ± 0.000 c | 0.46 ± 0.050 bB | 0.53 ± 0.003 aC | 0.00 ± 0.001 |
|                        | SB Skins  | 0.25 ± 0.003 bB | 0.39 ± 0.005 aA | 0.42 ± 0.005 bB | 0.78 ± 0.014 aA | 0.01 ± 0.001 |
|                        | SB Flesh  | 0.05 ± 0.002 c | 0.05 ± 0.001 c | 0.51 ± 0.007 bA | 0.71 ± 0.007 aB | 0.01 ± 0.001 |
| **Chlorogenic Acid (µg/g)** | BP1 Skins | 0.00 ± 0.001 | 0.00 ± 0.001 |
|                        | BP1 Flesh  | 0.00 ± 0.001 | 0.00 ± 0.001 |
|                        | SB Skins  | 0.00 ± 0.001 | 0.00 ± 0.001 |
|                        | SB Flesh  | 0.00 ± 0.001 | 0.00 ± 0.001 |

Values represent mean ± SD. Different small letters along the row per block represent significant differences at p < 0.05 and different capital letters down the column represent significant differences at p < 0.05. No heat was applied to the control. BP1 = Non-pigmented control; SB = Salad Blue.

3.2. Antimycobacterial Activity

The antimycobacterial activity of Solanum tuberosum (ethanol extracts) of both cultivars and treatments of BP1 and SB was investigated. The ethanolic extracts of all the tested samples did not show activity at the highest test concentration of 1000 µg/mL, as shown in Table 2. The positive drug control ciprofloxacin showed an MIC value of 0.325 µg/mL.

Table 2. Antimycobacterial activity against M. smegmatis (MIC µg/mL).

| Antimycobacterial Activity against M. smegmatis (MIC µg/mL) |
|-------------------------------------------------------------|
| Control SB | 24 °C SB | Control BP1 | 24 °C BP1 |
| NA | NA | NA | NA |

Ciprofloxacin | 0.325

NA—Not Active at the highest test concentration of 1000 µg/mL.

3.3. Antiproliferative Assay

The antiproliferative ethanolic extract activity of S. tuberosum L. cultivars SB and BP1 subjected to two RZTs was tested against HepG2 liver cells. The IC50 values of the samples ranged between 267.7 ± 36.17 µg/mL and >400 µg/mL, following 72 h of incubation as shown in Table 3. According to [28], after 72 h of incubation, plant extracts with IC50 values greater than 100 µg/mL are non-cytotoxic to the particular cell line. However, there is an increase in activity when SB and BP1 varieties are compared.
Table 3. Antiproliferative activity against Hepatocellular carcinoma cells (HepG2) (IC50 µg/mL).

| Control SB       | 267.7 ± 36.17 |
|------------------|---------------|
| 24 °C SB         | 290.8 ± 39.35 |
| Control BP1      | 393.0 ± 34.17 |
| 24 °C BP1        | NA            |

Controls

| Actinomycin      | 0.49 ± 15.91  |

NA—Not Active at the highest test concentration of 1000 µg/mL.

4. Discussion

4.1. Caffeic, Chlorogenic, and Ferulic acid Content in Solanum tuberosum L. Exposed to Higher Root Zone Temperature

The results of the current study confirmed what was seen in the study conducted previously by [29], which showed that chlorogenic acid concentration was significantly lower in yellow-fleshed potatoes in comparison with the high values reported in colored potatoes. Furthermore, an elevated RZT showed minimal chlorogenic acid concentration increase. Interestingly, caffeic acid concentration increased when SB and BP1 were exposed to a higher RZT. The concentration of chlorogenic acid in red- or purple-fleshed cultivars has previously been reported to be 2.2 to 3.5 times higher than in yellow- and white-fleshed cultivars [30]. Similar results have been reported by other authors, including [31–35]. Phenolic compounds have a direct function in the type of response given by the plant when exposed to stress, such as from sun exposure or pathogen infection [36]. This is, therefore, a direct indication as to why the increase in RZT has a direct effect on the concentration of the phenolic, as seen in the current study.

4.2. Antimycobacterial Activity

Many literature studies have shown that the potato contains a variety of phenolic acids as a means of protection against microbes, viruses, and insects [37]. The mechanism of action of the antimicrobial potential of phenolic compounds has been proposed to be through the destabilization and permeation of the membrane of the microbe, which results in changes to the efflux activity and polarization; in addition, virulence factors, such as hydrophobicity, are directly affected [29]. A study conducted by [38] showed that the phenolic compound myricetin showed low antimycobacterial inhibition against *M. smegmatis* with an MIC value of 32 mg/L. Another study conducted by [39] showed that chlorogenic acid showed no inhibition against *M. smegmatis* with an MIC value of >2500 µg/mL. Moreover, during this study, a direct correlation of phenolic content to antimycobacterial activity could not be shown [39]. Due to the high levels of chlorogenic acid found in both cultivars, it could be concluded that this might be why no inhibitory activity was found against *M. smegmatis*. Further studies based on previous literature could focus on the activity of chlorogenic acid against other Gram-negative and Gram-positive bacteria and microbes.

4.3. Antiproliferative Activity

Several studies have concluded that phenolics are important sources of antioxidants and that a diet rich in antioxidants can have remarkable effects on the risk of developing cardiovascular and neurodegenerative diseases including cancer and diabetes [40–43]. The anticancer activity of chlorogenic acid was investigated both in vitro and in vivo against the HepG2 cell line and HepG2 xenografts in nude mice. The study concluded that chlorogenic acid in greater concentrations had increased inhibition of HepG2 cells. The xenograft studies on nude mice achieved the same results and showed the suppression of the progression of the HepG2 xenograft [44]. Although there was no effective antiproliferative activity, as seen in the results against the HepG2 cell line by both the cultivars tested, it
should be noted that the increase in chlorogenic acid content in the SB cultivar showed increased antiproliferative activity when compared with BP1. It should also be emphasized that although chlorogenic acid is present in high amounts in both cultivars, it is not the only phenolic compound, or compound in general, that is present, and the synergistic effects of all compounds in *Solanum tuberosum* L. should be noted when looking at antiproliferative activity.

5. Conclusions

The pigmented potato tubers’ antioxidant capacity (through the presence of ferulic, chlorogenic, and caffeic acids), antiproliferative activity, and antimycobacterial activity are cultivar specific. In our study, increasing the RZT had a significant effect on caffeic and chlorogenic acid in the pigmented cultivar SB. The same effect was reported in the antiproliferative study. Our results may offer the opportunity to test the same and other cultivars of *Solanum tuberosum* against other cancer cell lines. These findings are of interest because they increase the availability of information on the experimental investigations of different cultivars found within Southern Africa.

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