Untargeted profiling of field cultivated bush tea \( (Athrixia\ phylicoides\ DC.) \) based on metabolite analysis

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**Abstract:** Bush tea \( (Athrixia\ phylicoides\ DC.) \) is an aromatic South African indigenous plant used for many decades as a health beverage and medicine. Several studies have extensively investigated wild bush tea’s secondary metabolites, but the entire profiling of cultivated bush tea’s metabolites is limited in the literature. Thus, the objective of this study was to profile cultivated bush tea metabolites using liquid chromatography-quadrupole time of flight-tandem mass spectrometry (LC-QTOF-MS). The 31 metabolites profiled included; benjaminamide, chlorogenic, chrysosplenetin, coumarin, 6Z-docosenamide, naringemin 7-O-β-D-glucoside, 5-p-coumaroylquinic acid, integrastatin A, lutetin 7-O-(6-O-malonyl-β-D-glucoside), 1,3-dicaffeoylquinic acid, magnoshinin, okanin, (2S)-5-hydroxy-7-methoxy-6,8-dimethylflavone, (9Z,12Z,15Z)-octadecatrienoic acid, 2′-deaminio-2′-hydroxy-6′-dehydroparomamine, O-butanoylcarmitine, myricitrin, goric acid, tetracenoxyin X, sakurain, n-tryptophan, linoleamide, laricitrin 7-monoglucoside, l-β-phenylalanine, l-proline, pheophytin A, pheophorbide A, Pi(18:0/20:4(8Z,11Z,14Z,17Z)), steardionic acid, and giberellin A14 aldehyde. These annotated metabolites included phenolics, flavonoids, and quinic acids, indicating that bush tea is rich in metabolites, which have a potential wide range of health benefits.

**Keywords:** *Athrixia phylicoides*; Metabolites; Bush tea; LC-QTOF-MS; Phytochemistry.

**Introduction**

The effects of consumption of herbal teas, which contain thousands of phytochemicals, on general health and relief of symptoms in severe diseases have been reported (1-4). The quality of tea is inevitably associated with the presence and concentration of phytochemicals (5-8), thus, the discovery of novel tea compounds has increased globally since about 80% of the world’s population depends on traditional medicines and herbal teas for primary health care (9).

Bush tea \( (Athrixia\ phylicoides\ DC.) \), an aromatic leafy shrub native to South Africa (10), holds an impressive history treating various ailments (11) and contributed to the primary health care of ancient people (12). Several studies have been conducted to assess bush tea’s potential as an herbal tea, and demonstrated that bush plant has phytochemicals and secondary metabolites, which have healing effects and pharmacological properties (13-16). However, these studies have only identified and characterized bush tea sampled from the wild. Metabolomics approaches such as untargeted ultraperformance liquid chromatography–mass spectrometry (UPLC-MS), targeted UPLC-MS, and untargeted \(^1\)H NMR have been recommended for tea phytochemical composition profiling to evaluate the tea quality (17). In bush tea, the whole profiling of cultivated bush tea’s metabolites using metabolomic analysis is still not investigated. In this study, cultivated bush tea metabolites were profiled using liquid chromatography-quadrupole
time of flight-tandem mass spectrometry (LC-QTOF-MS/MS).

Materials and Methods

Experimental site
The trial was carried out at the Agricultural Research Council Vegetable and Ornamental Plant Institute situated about 25 km north of central Pretoria on the Moloto/KwaMhlanga Road (R573), GPS coordinates 25° 59” S; 28° 35” E. The farm covers approximately 4000 ha, of which only 650 ha is under irrigation. The bush tea was grown under 40% shade net and drip irrigation at frequency of 2.5 mm/h. Agronomic practices such as fertilization was applied as per Tshivhandekano et al. (18) who recommended that 150 N kg/ha.

Sample preparation
Two-year mature leaf and twig samples were harvested from bush tea plants. Harvested leaves were air-dried at room temperature in the shade. Samples were then ground to a powder using a benchtop grinder and stored in glass vials below -50°C until extraction.

Extraction of bush tea samples for LC-MS
Ten milligrams of each sample were weighed into 2-mL Eppendorf tubes. One mL solvent (0.1% formic acid in methanol) was added to dissolve the samples and vortexed for 1 min then placed in a sonicator waterbath for 30 min. Samples were then centrifuged for 5 min at 10,000 rpm on a benchtop centrifuge. Approximately 700 μL of the supernatant were pipetted into HPLC vials and fitted with metal caps with rubber septa and secured with a crimper.

QTOF LC-MS analysis
The supernatants were subsequently injected into the QTOF LC-MS (Thermo Scientific, Dionex Ultimate 3000 Dionex Softron GmbH, Dornierstr. 4, Germany). Metabolites were separated using a gradient of H₂O with 0.1% formic acid (solvent A) and acetonitrile (solvent B), using a Dionex Ultimate 3000 UPLC at a flow rate 0.3 mL/min on a Waters BEH C18, 2.1 × 100 mm column. Mass spectrometry data was obtained on a QTOF Bruker Impact II (Bruker Daltonics GmbH, Bremen, Germany) using electron spray ionization (ESI) running in positive mode, that scans between 50-1600 m/z, with nebulizer at 1.8 bar and dry gas as 8 L/min.

Liquid chromatography-MS data were analyzed using the Compass data analysis tool version 4.3.110 and converted into buckets after peak integration and Pareto scaling. Peaks were identified according to actual mass, MS/MS and retention time (RT). Accurate mass and MS/MS spectral data were compared to the Kyoto Encyclopedia of Genes and Genomes (KEGG), Chemical Entities of Biological Interest (CheBI), Chemspider and MetFrag databases.

Results and Discussion
There is lack of distinct literature on compounds in cultivated bush tea. The previous studies reported compounds from bush tea that were harvested from the wild, which is considered unsustainable since the domestication of the wild plant is critical to avoid extirpation of this plant. The results from this study in Tables 1 and 2 demonstrate the 31 compounds detected from cultivated bush tea using LC-QTOF-MS. The compounds annotated were validated through fragment ions denoted in Table 1 and 2, and chromatogram in Figure 1. The selected extracted ion chromatograms of the cultivated bush tea extract included O-butanylocarnitine, l-β-phenylalanine, d-tryptophan, chlorogenate, coumarin, naringenin-7-O-β-d-glucoside, chrysospleninetin, steardonic acid, gibberellin A14 aldehyde, 9Z,12Z,15Z-octadecatrienoic acid, 6Z-docosenamide (Figure 1). About nine compounds were previously reported to be present in bush tea while 25 compounds are newly detected bush tea compounds (Table 3).

The secondary metabolites profiled included benzaminamide (mass 681.627; RT 8.85 min; C₄₂H₅₄NO₁₈) which was isolated from Ficus mucuso Welw. ex Ficalho and possess antimicrobial activities (19). Another secondary metabolite annotated chlorogenate or chlorogenic acid (mass 354.095; RT 4.61 min; C₁₇H₁₅O₉) was also profiled as one of the compounds in bush tea (Figure 2). This finding is consistent to with results reported by de Beer et al. (20) who reported the presence of chlorogenic acid in bush tea. Chlorogenate was also reported as a compound that lowers the risk of blood pressure (21). Chrysospleninetin (mass 374.107; RT 8.96 min; C₁₉H₁₉O₈), a secondary metabolite that was repor-
| Compound | Retention time (min) | Elemental composition | [M+H]+ | Frag ion 1 | Frag ion 2 | Frag ion 3 | Frag ion 4 |
|----------|---------------------|-----------------------|--------|-----------|-----------|-----------|-----------|
| Benjaminamide | 8.85 | C₁₇H₂₁NO₆ | 681.627 | 298.2740 |
| Chlorogonate | 4.61 | C₁₂H₁₇O₉ | 354.0951 | 117.0335 | 135.0441 | 145.0284 | 163.0390 |
| Chryso sple nitin | 8.96 | C₁₅H₂₀O₉ | 374.1072 | 135.0441 | 299.0550 | 302.0421 | 317.0655 |
| Coumarin | 5.16 | C₉H₈O₂ | 146.037 | 91.0542 | 119.0491 |
| 6Z-Docosenamide | 11.93 | C₁₈H₃₀NO | 337.334 | 71.0542 | 85.0102 | 86.0600 | 97.0648 |
| Naringenin 7-O-β-d-glucoside | 8.70 | C₁₇H₂₃O₁₀ | 434.121 | 362.0632 | 377.0867 | 405.0816 |
| 5-p-Coumaroylquinic acid | 4.21 | C₁₇H₂₁O₈ | 338.108 | 91.0542 | 119.0491 | 147.0441 |
| Integastatin A | 7.25 | C₂₈H₃₉O₉ | 332.0526 | 347.0706 | 375.0710 | 405.0816 |
| Luteolin 7-O-(6-O-malonyl-β-d-glucoside) | 6.29 | C₁₆H₂₀O₄ | 534.101 | 135.0441 | 145.0284 | 163.0389 |
| 1,3-Dia cetoxyquinic acid | 5.18 | C₂₀H₂₂O₂ | 516.126 | 135.0441 | 145.0284 | 163.0390 | 499.1234 |
| Magnoshinin | 8.85 | C₁₇H₂₃O₈ | 414.204 | 119.0491 | 135.0804 | 135.0804 | 135.0804 |
| Okanin | 6.55 | C₁₅H₂₃O₆ | 288.063 | 153.0182 | 163.0389 | 163.0390 | 163.0390 |
| (2S)-5-Hydroxy-7-methoxy-6,8-dimethylflavanone | 9.00 | C₁₇H₂₃O₆ | 298.121 | 107.0491 | 119.0491 | 135.0804 | 147.0441 |
| (9Z,12Z,15Z)-Octadecatrienoic acid | 10.18 | C₁₈H₃₀O₂ | 278.225 | 81.0699 | 95.0855 | 95.0855 | 123.1168 |
| 2″-Deamino-2″-hydroxy-6″-dehydroparomamine | 6.48 | C₁₁H₂₁NO₂ | 322.138 | |
| O-Butanoylcarnitine | 3.09 | C₁₁H₂₁NO₂ | 231.147 | 214.1438 |
| Myricitrin | 6.90 | C₂₁H₂₀O₁₂ | 464.095 | 303.0499 |
| Gorlic acid | 10.18 | C₁₈H₃₀O₂ | 278.225 | 67.0542 | 81.0699 | 95.0855 | 123.1168 |
| Tetracenomycin X | 8.18 | C₁₅H₁₉NO₂ | 484.101 | 119.0491 | 147.0441 | 147.0441 | 147.0441 |
| Sakuranin | 6.36 | C₂₂H₂₄O₁₀ | 448.137 | 419.0972 |
| N-(2-Hydroxyheptadecanoyl)-1-O-β-d-glucosyl-15-methylhexadecasphing-4-enine | 5.52 | C₄₀H₇₇NO₉ | 301.049 | 536.5037 | 554.5143 |

Table 1. LC/Q-TOF-MS exact masses for cultivated bush tea secondary metabolites and their main fragment ions.

| Compound | Retention time (min) | Element composition | [M+H]+ | Frag ion 1 | Frag ion 2 | Frag ion 3 | Frag ion 4 |
|----------|---------------------|---------------------|--------|-----------|-----------|-----------|-----------|
| d-Tryptophan | 4.13 | C₁₁H₁₂N₂O₂ | 204.093 | 91.0542 | 118.0541 | 132.0808 | 144.0808 |
| Linoleamide | 3.83 | C₁₈H₃₃NO | 279.256 | 83.0491 | 83.0855 | 186.0600 |
| Laricitrin 7-monoglucoside | 8.26 | C₁₁H₁₉NO₂ | 494.106 | 333.0605 | 318.037 | 85.0824 |
| L-β-Phenylalanine | 3.33 | C₁₉H₂₁NO₂ | 165.079 | 120.0808 |
| L-Proline | 9.22 | C₉H₁₈NO₂ | 115.063 | 70.0651 |
| Pheophytin A | 11.25 | C₁₉H₂₁NO₄ | 870.569 | 533.2547 | 533.2546 | 593.2758 |
| Pheophorbide A | 11.57 | C₁₉H₂₁NO₄ | 592.2686 | 461.2336 | 461.2335 |
| PI(18:0/20:4(8Z,11Z,14Z,17Z)) | 12.46 | C₁₉H₂₁NO₂ | 886.5571 | 549.2459 | 591.2564 | 609.2670 |
| Stearidonic acid | 9.94 | C₁₈H₂₈O₂ | 276.209 | 93.0699 | 109.1012 | 135.1168 | 149.1325 |
| Gibberellin A14 aldehyde | 9.38 | C₁₇H₂₀O₄ | 332.199 | 127.0389 | 287.2006 |

Table 2. LC/Q-TOF-MS exact masses for the cultivated bush tea metabolites and their main fragment ions.
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Table 3. Compounds previously found in bush tea and newly annotated.

| Novel compounds found in this study | Compounds found previously in wild bush tea | References |
|------------------------------------|------------------------------------------|------------|
| Benjaminnamide                     | Chlorogenate                              | (16, 20)   |
|                                    | Chrysosplenetin                           | (25)       |
|                                    | Coumarin                                 | (23)       |
| 6Z-Docosenamide                    | Naringenin 7-O-beta-d-glucoside           | (25)       |
|                                    | 5-p-Coumaroylquinic acid                  | (16)       |
| Integrastatin A                    |                                         |            |
| Luteolin 7-O-(6-O-malonyl-beta-d-glucoside) | 1,3-Dicaffeoylquinic acid                |            |
| Magnoshinin                        |                                         |            |
| Okainin                            |                                         |            |
| PI(18:0/20:4(8Z,11Z,14Z,17Z)       |                                         |            |
| (2S)-5-Hydroxy-7-methoxy-6,8-dimethylflavanone |                     |            |
| (9Z,12Z,15Z)-Octadecatrienoic acid |                                         |            |
| Gorlic acid                        |                                         |            |
| Sakuranin                          |                                         |            |
| Tetracenomycin X                   |                                         |            |
| D-Tryptophan                       |                                         |            |
| 2”-Deamino-2”-hydroxy-6”-dehydroaromamine |                                         |            |
| O-Butanoylcaritnine                |                                         |            |
| Linoleamide                        |                                         |            |
| N-(2-Hydroxyheptadecanoyl)-1-O-beta-d-glucosyl-15-methylhexadecasphing-4-ene | | |
| Laricitrin 7-monogluscoside        |                                         |            |
| t-Phenylalanine                    |                                         | (25)       |
| t-Proline                          |                                         | (25)       |
| Pheophytin A                       |                                         |            |
| Pheophorbide A                     |                                         |            |
| Steardionic acid                   |                                         |            |
| Gibberellin A14 aldehyde           |                                         |            |

...ted to have been isolated from the root of *Berneuxia thibetica* Decne. and possesses an anti-viral activity (22), was also annotated from bush tea extracts in this study. Consistent with Padayachee (23), a phenylpropa-noicoumarin (mass 146.037; RT 5.16 min; C<sub>11</sub>H<sub>13</sub>O<sub>2</sub>) was present in bush tea and this secondary metabolite was reported to play a significant role in the sweet and aromatic odor quality of green tea (24) and possesses anti-microbial properties. Bush tea extract from this study had a flavonone glycoside naringenin-7-O-beta-d-glucoside (mass 434.121; RT 8.70 min; C<sub>21</sub>H<sub>22</sub>O<sub>10</sub>) also known as prunin (Figure 2), this metabolite was detected in wild bush tea (25). A polyphenolic acid, 5-p-coumaroylquinic acid (mass 338.108; RT 6.48 min; C<sub>16</sub>H<sub>18</sub>O<sub>8</sub>), a compound commonly found in teas and fruits (26) was detected.

Bush tea extracts in this study also contained an antiviral compound, integrastatin A (mass 332.0526; RT 7.25 min; C<sub>20</sub>H<sub>20</sub>O<sub>9</sub>) also found in bush tea. This study also reported the presence of luteolin 7-O-beta-d-glucoside (mass 534.101; RT 6.29 min; C<sub>28</sub>H<sub>22</sub>O<sub>11</sub>), a plant flavonoid that possesses antioxidant, antiviral, and antibacterial properties (27). A caffeoylquinic acid (CQA), the main constituent of chlorogenic acid, 1,3-dicaffeoylquinic acid (mass 516.127; RT 5.18 min; C<sub>25</sub>H<sub>24</sub>O<sub>12</sub>), was also found in bush tea extract.

This study showed that bush tea also contains a neolignan, an anti-inflammatory compound magnoshinin (mass 414.204; RT 8.85 min; C<sub>25</sub>H<sub>25</sub>O<sub>12</sub>) (28). A chalconoid compound, okanin (mass 288.063; RT 6.55 min; C<sub>15</sub>H<sub>12</sub>O<sub>6</sub>) was annotated and it possess antimicrobial and anti-inflammatory properties (29). Bush tea extract in this study contained a flavonone, (2S)-5-hydroxy-7-methoxy-6,8-dimethylflavanone (mass 298.121; RT 5.16 min; C<sub>12</sub>H<sub>20</sub>O<sub>5</sub>), was detected. A flavonoid, myricitrin (mass 464.095; RT 6.90 min; C<sub>21</sub>H<sub>20</sub>O<sub>12</sub>), was also found in cultivated bush tea extract (Figure 2). This compound is a nitric oxide and protein kinase C inhibitor, which possesses antipsychotic-like and anxiolytic-like effects in animal models of psychosis and anxiety (30). Gorlic acid (mass 278.225; RT 10.18 min; C<sub>18</sub>H<sub>30</sub>O<sub>2</sub>) was detected. The study also showed that bush tea extracts contain a...
flavonone compound called sakuranin (mass 448.137; RT 6.36 min; \( \text{C}_{22}\text{H}_{24}\text{O}_{10} \)), which exhibits antioxidant activity (31).

The primary metabolites annotated in this study included a compound n-tryptophan (mass 204.093; RT 4.13 min; \( \text{C}_{11}\text{H}_{20}\text{N}_{2}\text{O} \)), which is an \( \alpha \)-amino acid. L-tryptophan (mass 279.256; RT 3.83 min; \( \text{C}_{11}\text{H}_{13}\text{NO} \)), a fatty amide lipid molecule was also detected from bush tea extracts. A lipid, lacticin 7-monoglucoside (mass 494.106; RT 8.26 min; \( \text{C}_{22}\text{H}_{20}\text{O}_{10} \)), was also detected. An essential amino acid, L-\( \beta \)-phenylalanine (mass 165.079; RT 3.33 min; \( \text{C}_{11}\text{H}_{12}\text{NO} \)), was found in bush tea extract. The amino acid L-proline (mass 115.063; RT 9.22 min; \( \text{C}_{2}\text{H}_{4}\text{NO} \)) was present in cultivated bush tea extract. A chlorophyll compound, phophytoxin a (mass 870.565; RT 11.25 min; \( \text{C}_{55}\text{H}_{35}\text{NO}_{2} \)), was also profiled (Figure 2) and it has been reported to possess antimicrobial, antioxidant and anti-aging effect (32).

Consistent with Malongane and McGaw and Nyoni and Mudau (25) phophorhbia A (mass 592.2686; RT 11.57 min; \( \text{C}_{29}\text{H}_{30}\text{NO}_{2} \)), a product of chlorophyll breakdown, which was found to possess anticancer activity (33), was also present in bush tea. An acidic phosphatidyl-

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**Interest conflict**

The authors declare no conflict of interest.
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