

1 **Comparative Profiling of Bioactive Compounds and Antioxidant Activity of Extracts from**
2 **Selected Medicinal Plants: Implications for Mitigating Obesity-related Inflammation**

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21
22 **Abstract**

23
24 Obesity is a metabolic disorder, contributing to various health complications, including diabetes,
25 hypertension, and cardiovascular dysfunction. Increased use of plant extracts to reduce obesity risk
26 reflects consumer preference for natural remedies and scientific validation for their safety and
27 efficacy. This study profiled bioactive compounds in methanolic extracts from the leaves and roots
28 of *Merwillia plumbea* (Lindl.) Speta, *Hypoxis hemerocallidea* Fisch, *Eucomis autumnalis* (Mill.)
29 Chitt, and *Pentanisia prunelloides* (Klotzsch) Walp. The objective was to explore and compare
30 the medicinal properties of distinct plant parts for their potential to mitigate obesity-induced

31 inflammation. *P. prunelloides* leaves and roots had higher concentrations of phenolics (123.92
32 mg/mL and 110.01 mg/mL) and flavonoids (44.4 mg/mL and 55.05 mg/mL), respectively.
33 Gallotannins were significantly higher in *H. hemerocallidea* roots (5.19 mg/mL) while
34 proanthocyanidins were predominantly found in *P. prunelloides* roots (35.77 mg/mL). The
35 antioxidant activity was assessed by ferric reducing antioxidant potential (FRAP) and DPPH
36 radical scavenging activity (RSA) assays. *P. prunelloides* roots had higher FRAP (2.97 mg/mL)
37 and moderate DPPH (RSA) (52.89%) while *M. plumbea* roots had the highest DPPH RSA
38 (80.86%) and lower FRAP (2.25 mg/mL). *E. autumnalis* roots and leaves showed FRAP values of
39 2.78 and 2.13 mg/mL, and DPPH RSA of 80.72 and 74.54%, respectively. The results revealed
40 that all plants investigated had considerable amounts of bioactive compounds with *P. prunelloides*
41 showing the highest concentration, highlighting its potential for further pharmaceutical and
42 nutraceutical exploration. Further research validating the bioactivity of key compounds in vivo,
43 exploring seasonal variations, and assessing optimal harvesting practices is paramount for the
44 sustainable utilization of these medicinal plants.

45

46 **Keywords:** adipose, antioxidants, inflammation, inhibition, phytochemicals.

47

48 1. Introduction

49 Obesity has become a pandemic, presenting a burgeoning challenge due to its intricate and chronic
50 nature, influenced by the contribution of myarid factors. This condition involves disruptions in
51 multiple physiological aspects, such as imbalances in energy, dysregulation of central appetite,
52 hormone changes, and gut microbiome composition (Breton et al., 2022). Recent global data
53 reported by the World Health Organization (WHO) revealed that more than 1 billion individuals,
54 comprising 650 million adults, 340 million adolescents, and 39 million children, are affected by
55 obesity (Aborode et al., 2023). Survey data from 2016 underscores the severity of the issue,
56 indicating that 68% of women and 33% of men in South Africa were categorized as obese,
57 surpassing prevalence rates in the sub-Saharan region (NDoH and SAMRC, 2017, Smith et al.,
58 2023).

59

60 Obesity is characterized by adipogenesis, a process encompassing hyperplasia and hypertrophy
61 as body adaptive response mechanisms to excess consumption of calories (Rosen and Spiegelman,

62 2014). Obesity is also linked with low-grade inflammation, marked by the infiltration of
63 macrophages and the secretion of pro-inflammatory cytokines, such as interleukin 6 (IL-6),
64 interleukin-1 β (IL-1 β), and tumour necrosis factor (TNF- α) (Chen et al., 2018). Prolonged
65 activation of inflammatory cytokines inhibits anti-inflammatory response, resulting in oxidative
66 stress, a pathological state characterized by a dysregulation between the generation of reactive
67 oxygen species (ROS) and the body's antioxidant defence systems. This oxidative imbalance
68 exacerbates insulin resistance, the development of metabolic disorders, and increases the risk of
69 conditions such as diabetes mellitus, heart failure, dyslipidemia, and fatty liver (Leyva-Jiménez et
70 al., 2020, Lim et al., 2013).

71
72 Antioxidants play an important role in balancing ROS, reducing lipid peroxidation, and
73 modulating inflammatory signaling pathways (Zielinska-Blizniewska et al., 2019). Plant-derived
74 antioxidants, particularly polyphenolic compounds have gained increasing attention owing to their
75 capacity to scavenge free radicals, modulate adipogenesis, and enhance metabolic homeostasis.
76 Among the most studied plant-derived antioxidants, several bioactive compounds have
77 demonstrated anti-inflammatory and antioxidant effects in obesity management, including,
78 chlorogenic acid which inhibits pancreatic lipase by acting directly on its active site thereby
79 reducing dietary fat absorption (Podsędek et al., 2020). Catechin and proanthocyanidin reduce
80 adipogenesis by downregulating the expression of transcription factors, peroxisome proliferator-
81 activated receptor gamma (PPAR γ), and CCAAT/enhancer-binding protein alpha (C/EBP α) which
82 are essential for the differentiation of preadipocytes into mature adipocytes (Kim et al., 2024,
83 Nugrahini et al., 2024). Quercetin also exerts an anti-obesity effect by inhibiting sterol regulatory
84 element-binding protein 1c (SREBP-1c) involved in the synthesis of fatty acids in the liver
85 (Oliveira et al., 2022). Gallotannins block the active site of pancreatic lipase to reduce fat
86 absorption and reduce adipogenesis by decreasing the expression of PPAR γ and C/EBP α (Singh
87 et al., 2020).

88
89 Traditional medicinal plants have been extensively examined for their polyphenolic constituents
90 and antioxidant potential, particularly in alleviating oxidative stress and inflammation. Several
91 plants including, *Merwillia plumbea*, *Hypoxis hemerocallidea*, *Eucomis autumnalis*, and
92 *Pentanisia prunelloides*, have been utilized as natural remedies for their potential as antioxidant,

93 metabolic regulatory properties and anti-inflammatory (Alaribe et al., 2018, Matyanga et al., 2020,
94 Mpofo et al., 2014). However, despite their traditional applications, limited research has been
95 conducted to systematically profile their bioactive compounds and antioxidant activities in the
96 context of obesity-induced inflammation. Additionally, it remains unclear which plant parts
97 harbour high concentrations of bioactive compounds, making it essential to investigate their
98 specific phytochemical composition and functional potential. This study aims to address these gaps
99 by profiling the phytochemical and antioxidant activities of *M. plumbea*, *H. hemerocallidea*, *E.*
100 *autumnalis*, and *P. prunelloides*, with a focus on their potential therapeutic benefits against
101 obesity-induced inflammation.

102 **2. Materials and methods**

103 **2.1 Plant material**

104
105 The plant samples of *M. plumbea*, *H. hemerocallidea*, *E. autumnalis*, and *P. prunelloides* were
106 collected from the University of KwaZulu-Natal Botanical Garden, Pietermaritzburg, South Africa
107 (29°37'28" S30°24'13" E). The plant species was identified by a qualified horticulturist Mrs
108 Alison Young. A voucher specimen of the plant was deposited at the Bews herbarium, School of
109 Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa The plant samples
110 were separated into roots and leaves and rinsed with water to remove debris. The samples were
111 thereafter oven-dried (Incotherm, Laboratory Consumables Pty, South Africa) at 60 °C for 3 days
112 as described by Ncube et al. (2011).

113 **2.2 Experimental design**

114
115 The experiment was conducted using a factorial randomised complete design (RCD) to evaluate
116 the effects of plant species and parts on the bioactive compound composition and antioxidant
117 activity of medicinal plant extracts. The study included four plant species namely, *M. plumbea*, *H.*
118 *hemerocallidea*, *E. autumnalis*, and *P. prunelloides*. Each plant species was evaluated based on
119 two plant parts (leaves and roots), leading to a factorial structure (4 plant species and 2 plant parts).
120 Each treatment (species × plant part) was replicated three times, resulting in 24 experimental units
121 (4 species × 2 plant parts × 3 replicates = 24n).

122 **2.3 Preparation of plant extracts**

123
124 Dried plants parts of *M. plumbea*, *H. hemerocallidea*, *E. autumnalis*, and *P. prunelloides* (leaves
125 and root) material were ground into a fine powder using an electric warring. A 2 g of powdered
126 plant material was extracted separately in 40 mL of 80% (v/v) methanol and distilled water as
127 described by Yildiz-Ozturk et al. (2015). The setup was placed on a Labcorn Platform shaker
128 (Laboratory consumables, Durban, South Africa) at 150 rpm at 25 °C for 24 h. The plant extracts
129 were centrifuged at 3000 rpm for 5 min and filtered through Whatman No.1 filter paper (Whatman,
130 UK) to obtain a homogenous mixture. Filtrates were stored in a refrigerator at 4 °C until use. All
131 extractions were performed in triplicate to ensure statistical reliability of the results.

132 **2.4 Determination and quantification of total phenolic compounds**

133
134 The concentration of total phenolics was determined using the Folin Ciocalteu (Folin C) assay as
135 described by Joint (2000). A 50 µL of plant extract was transferred into a test tube, followed by
136 adding 950 µL of distilled water, 500 µL of 1 N Folin C phenol reagent, and 2.5 mL of 2% (w/v)
137 sodium carbonate. A blank containing 80% (v/v) aqueous methanol was prepared. The mixtures
138 were incubated at room temperature for 40 min. The absorbance was read at 725 nm using a UV–
139 vis spectrophotometer (Thermoscientific, Multiskan Sky, Vantaa, Finland). The total phenolic
140 concentrations were expressed as gallic acid equivalents at a concentration from 2-150 mg/mL (y
141 $= 0.0151x + 0.0519$; $R^2 = 98$). All measurements were performed in triplicate.

142 **2.5 Extraction and quantification of flavonoids**

143
144 The 50 µL plant extract was added to 1 mL methanol in a test tube followed by adding 2.5 mL
145 methanolic–1 N hydrochloric acid (95:5, v/v) and 2.5 mL vanillin reagent (1 g/100 mL acetic acid)
146 (Csepregi et al., 2013). The blank containing 80% (v/v) methanol was prepared. The mixture was
147 incubated for 20 min at room temperature, absorbance was read at 500 nm using a UV-vis
148 spectrophotometer (Thermoscientific, Multiskan Sky, Vantaa, Finland). The flavonoid
149 concentration was quantified from the standard curve ranging from 18 to 150 mg/mL ($y = 0.0005x$
150 $- 0.0057$; $R^2 = 96$) expressed as catechin equivalents (Hagerman, 2002). All measurements were
151 conducted in triplicate to ensure reproducibility and statistical reliability of the results.

152 **2.6 Extraction and quantification of gallotannins**

153 Plant extracts (50 µL) in a test tube were added up to 1 mL with distilled water and followed by
154 100 µL of 0.4 N sulphuric acid and 600 µL of 0.67% (w/v) rhodanine methanolic solution was
155 added to the diluted extracts (Moyo et al., 2013). After 5 min, 200 µL of 0.5 N potassium hydroxide
156 was added followed by 4 mL distilled water after a further 2.5 min. The mixtures were left for an
157 additional 15 min at room temperature, after which the absorbance at 520 nm was read using a
158 UV–vis spectrophotometer (Thermoscientific, Multiskan Sky, Vantaa, Finland) against a blank
159 that contained methanol instead of plant extract. Gallotannin concentration was interpolated from
160 the 1.2 – 75mg/mL standard curve ($y = 0.0226x - 0.0073$; $R^2 = 99$) and expressed as gallic acid
161 equivalents Joint (2000). Each sample was analysed in triplicate, ensuring the accuracy and
162 repeatability of the results.

163 **2.7 Determination of proanthocyanidins**

164 The 1.2 mL of butanol–Hydrochloric acid reagent (95:5, v/v) was transferred to a test tube
165 containing 200 µL of each plant extract sample and followed by 40 µL ferric reagent (2% ferric
166 ammonium sulphate in 2 N Hydrochloric acid) (Ghuman et al., 2016). The test combination was
167 vortexed (Lab dancer V, Cole Parmer, Germany) and placed in a boiling water bath for 1 h. The
168 absorbance was read at 580 nm using a UV–vis spectrophotometer (Thermoscientific, Multiskan
169 Sky, Vantaa, Finland). All measurements were made in triplicate. A blank containing 80% aqueous
170 methanol was also prepared but without heating. Condensed tannin (%) was calculated as
171 leucocyanidin equivalents using Eq. 1, developed by Porter et al. (1985).

172

$$173 \text{ Condensed tannin (\%)} = (A_{580 \text{ nm}} \times 78.26 \times \text{Dilution factor}) / (\% \text{ dry matter}) \quad 1$$

174

175 This formula assumes that the effective $E^{1\%, 1 \text{ cm}, 580 \text{ nm}}$ of leucocyanidin is 460 (Al-Alimi et al.,
176 2017). The dilution factor was 1 since 80% methanol was not added to prevent the absorbance
177 from exceeding 0.6. If absorbance exceeds 0.6, the extraction solvent dilutes it to allow the
178 spectrophotometer to read the sample.

179 **2.8 Determination of antioxidant activity using ferric-reducing antioxidant potential (FRAP)**

180 The FRAP assay was measured using a modified method described by Rabeta and Faraniza (2013).
181 Briefly, 90 µL of plant extract was transferred into a tube and followed by 40 µL of potassium
182 phosphate buffer having a pH of 7.2. Potassium ferricyanide (40 µL) containing 1% (w/v) was
183 added, covered with aluminium foil, and incubated at 50 °C for 20 min. After incubation, 120 µL
184 of distilled water was added, followed by 40 µL trichloroacetic acid (10 % (w/v)) and 30 µL of
185 0.1 % w/v FeCl₃ (prepared in dark room). The aqueous methanol (80%) was used as a blank. The
186 mixture was allowed to stand at room temperature for 30 min. The absorbance was measured at
187 593 nm using a UV–vis spectrophotometer (Thermoscientific, Multiskan Sky, Vantaa, Finland,
188 Vantaa, Finland). The standard calibration curve was constructed as described by Gohari et al.
189 (2011) using FeSO₄, 7H₂O at concentrations from 0.78 – 10mg.mL⁻¹ ($y = 0.7401x + 0.0405$; $R^2 =$
190 99). Analyses were made in triplicate.

191 **2.9 The antioxidant activity in scavenging DPPH**

192 Free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined using a
193 modified method described by Gulcin and Alwasel (2023), with all tests performed in triplicate.
194 Briefly, 20 µL of each plant extract was added in a test tube containing 130 µL of methanol and
195 followed by 1500 µL DPPH solution mixture. The reactions were performed under a dim light and
196 incubated at room temperature for 30 min. The absorbance was measured at 517 nm using a UV–
197 vis spectrophotometer (Thermoscientific, Multiskan Sky, Vantaa, Finland). Free radical
198 scavenging activity (RSA) was calculated using Eq. 2.

199

$$200 \text{ RSA\%} = (AD - AE / AD) \times 100$$

2

201

202 Where RSA% was the inhibition percentage of free radical scavenging activity, AD was a control
203 absorbance where the solvent was mixed with DPPH radical solution instead of plant extract and
204 AE was the absorbance of the tested sample.

205 **2.10 Profiling of phenolic compounds**

206 **2.10.1 Sample preparation**

207 A 0.5 g dry powdered plant material was extracted with 10 mL of 50% methanol/1 % formic acid,
208 concentrated by sonication for 1 h. The solution was centrifuged for 10 min at a speed of 3000
209 rpm, and the supernatant was diluted 5-fold with 50% methanol/0.1 % formic acid and transferred
210 to a glass vial for analysis. All extractions were performed in triplicate.

211 **2.10.2 Liquid chromatography-mass spectrometry (LCMS) analysis**

212 The analysis was conducted using a high-resolution Waters Cyclic Quadrupole time-of-flight
213 (qTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid
214 chromatograph (UPLC) (Waters, Milford, MA, USA). The column eluate first traversed a
215 Photodiode Array (PDA) detector before reaching the mass spectrometer, enabling simultaneous
216 collection of UV and MS spectra. The positive mode of electrospray ionization utilized a cone
217 voltage of 15 V, desolvation temperature of 275 °C, desolvation gas flow rate set at 650 L/h, and
218 the remaining MS parameters were fine-tuned for optimal resolution and sensitivity. Data were
219 detected by scanning from mass-to-charge ion (m/z) of 100 to 1500 in a resolution mode as well
220 as in mass spectrometry elevated energy (MSE) mode. Two channels of MS data were acquired in
221 MSE mode, one at a low collision energy (4 V) and the second one at 40 –100 V to obtain
222 fragmentation data. For precise mass determination, leucine enkaphalin was used as lock mass
223 (reference mass) and the instrument was calibrated with sodium formate. Separation was obtained
224 on a Waters HSS T3, 2.1 × 150 mm, 1.7 µm column. The 0.5 µL of 0.1% formic acid (solvent A)
225 was injected and followed by 0.5 µL acetonitrile containing 0.1% formic acid as solvent B. The
226 gradient was initiated at 100% solvent A for 1 min and reduced to 28% B over 11 min in a linear
227 way. It then increased to 40% B over 50 s and a wash step of 1.5 min at 100% B, followed by re-
228 equilibration to initial conditions for 2 min. The flow rate was 0.3 mL/min, and the column
229 temperature was kept at 60 °C. Compounds were quantified relatively against a calibration curve
230 established by injecting a range of rutin standards from 0.2 to 5 mg/L rutin.

231 **2.11 Data analysis**

232

233 All data collected from different plant species and parts were replicated three times and analysed
234 using a two-way ANOVA in GenStat [23rd Edition (VSN International, Hemel Hempstead, UK)].
235 Mean values recorded were separated using Duncan's multiple range test at a 5% significance
236 level. The Standard Error (SE) of the mean was calculated based on replication to represent the
237 precision of the mean values. Multivariate analysis and principal component (PCA) were executed
238 using MetaboAnalyst software version 6 (R package optiLCMS, 2024).

239

240 **3. Results and discussion**

241 **3.1 Phytochemical analysis**

242 **3.1.2 Phenolic content**

243

244 The analyses of phenolics, flavonoids, gallotannins, and proanthocyanidins revealed significant
245 differences ($p < 0.001$) among the plant species and parts. Phenolic concentrations were highest in
246 *P. prunelloides* leaves (123.92 mg/mL) and roots (110.01 mg/mL), followed by *H. hemerocallidea*
247 roots (89.21 mg/mL), with the lowest levels observed in *E. autumnalis* leaves (11.29 mg/mL) (Fig.
248 1A). The biosynthesis of phenolics is stimulated by the gene encoding for phenylalanine ammonia-
249 lyase (PAL) enzyme through shikimate and phenylpropanoid pathways (Deng and Lu, 2017). The
250 type and expression level of the PAL encoding genes differs with plant species. The increase in
251 the PAL enzymes may be attributed to higher phenolic concentration in these plant species. The
252 observed results showed significant differences ($p < 0.001$) between leaves and roots of the same
253 species where *P. prunelloides* exhibited 11% higher phenolic concentration in leaves compared to
254 roots. Similarly, *M. plumbea* showed a 49% increase in phenolic concentration in leaves than roots.
255 *E. autumnalis* also showed 33% higher phenolic in plant leaves than roots. The leaves are primarily
256 essential for photosynthesis, where light energy is converted into chemical energy. During this
257 process, a wide range of metabolites such as phenolic compounds are produced which are essential
258 for protecting the plant against UV radiation, pathogens, and herbivores (Chowdhary et al., 2021).
259 It could be argued that the higher phenolic concentration in the leaves of these plants was due to
260 the direct exposure of leaves to sunlight which necessitated their production to protect the plant
261 from UV radiation. Contrarily, *Hypoxis hemerocallidea* had a 63% higher phenolic content in roots

262 than the leaves, which we hypothesised to be a plant strategy to protect storage organs from
263 oxidative damage, pathogens, and other stresses during dormancy periods.

264

265 **3.1.2 Flavonoid content**

266

267 The higher flavonoid concentration was demonstrated by *P. prunelloides* roots (55.05 mg/mL) and
268 leaves (44.37 mg/mL) and *H. hemerocallidea* roots (44.6 mg/mL). A lower flavonoid was
269 observed in *E. autumnalis* (10.02 mg/mL) (Fig. 1B). The biosynthesis of flavonoid in plants is
270 associated with phenylpropanoid pathways through the synthesis of chalcone synthase (CHS),
271 chalcone isomerase (CHI), and flavanone 3-hydroxylase (F3H) (Kausar et al., 2020). The
272 regulation of these enzymes can result in differences in flavonoid concentration. Higher flavonoid
273 concentration in these plants may result from increased activities of these enzymes, conversely,
274 plant species that showed lower flavonoids explained lower activity levels of enzymes. Among
275 plant parts, *H. hemerocallidea* showed 56% higher flavonoid concentration in the roots than in
276 leaves, whereas other plant species exhibited no significant difference between plant parts. The
277 essential biochemical pathways for flavonoid biosynthesis in roots and leaves are the same,
278 however, regulation of these pathways can vary due to developmental cues, tissue-specific
279 metabolic needs, and environmental factors (Shi et al., 2024). For example, roots are the storage
280 organ that stores nutrients and energy for plant survival during adverse conditions, thus, flavonoids
281 possess antioxidant properties to protect the stored nutrients from microbial attack, oxidation, and
282 other stresses.

283 **3.1.3 Gallotannin content**

284

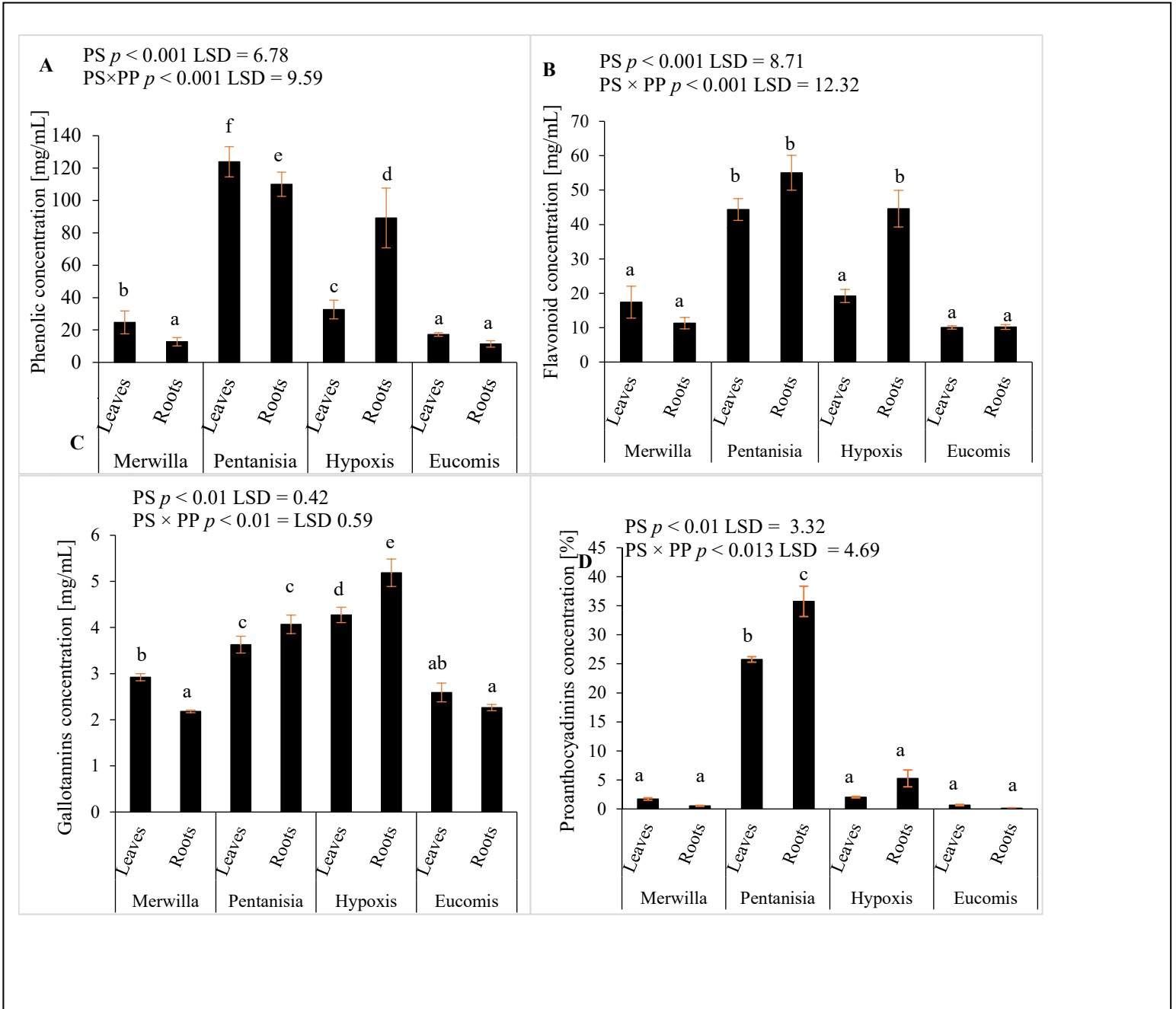
285 The quantification of gallotannin exhibited significant differences ($p < 0.001$) among various plant
286 species. *H. hemerocallidea* roots and leaves and *P. prunelloides* roots had higher gallotannin
287 concentrations (5.19 mg/mL, 4.27 mg/mL, and 4.07 mg/mL), respectively. The least gallotannin
288 was observed in *M. plumbea* roots (2.18 mg/mL). Similarly, Ncube et al. (2011) reported high
289 gallotannins in *H. hemerocallidea* than *M. plumbea*. Gallotannins are hydrolyzable tannins derived
290 from gallic acid, which is produced through the shikimate pathway. This pathway involves the
291 conversion of glucose and other precursors into galloyl glucose and consequently into gallotannins

292 via the action of the enzyme tannin acyl hydrolase (Sharma et al., 2017). Variations in gallotannin
293 concentration of these plant species were attributed by the activity of these enzymes. On the other
294 hand, differences in the genetics of these plant species also contribute to the variation of secondary
295 metabolites biosynthesis. Comparisons on plant parts showed significant variation, where *H.*
296 *hemerocallidea* (10%) and *P. prunelloides* (6%) had higher gallotannins in the roots than leaves
297 (Fig. 1C). On the other hand, *M. plumbea* (15%) and *E. autumnalis* (7%) exhibited higher
298 gallotannin content in the leaves than in the roots. Variation in gallotannin distribution between
299 roots and leaves among these plant species highlights their distinct strategies to mitigate
300 environmental stress such as UV radiation, herbivory, pathogen attack, and oxidative damage
301 (Alamgir, 2018).

302 **3.1.4 Proanthocyanidins content**

303
304 Proanthocyanidins are an essential class of structurally complex secondary metabolites and exist
305 naturally as phenolic compounds (Jiang et al., 2015). The results showed a significant difference
306 ($p \leq 0.001$) in proanthocyanidins among the tested plant species (Fig. 1D). A higher concentration
307 of proanthocyanidins was detected in *Pentanisia prunelloides* roots (36%) and leaves (26%) while
308 lower proanthocyanidins exhibited by *Eucomis autumnalis* (0.4%). Proanthocyanidins are
309 oligomers and polymers composed of elementary flavan-3-ol units particularly (-)-epicatechin and
310 (+)-catechin. This compound synthesis from L-phenylalanine through phenylpropanoid and
311 flavonoid pathways (Mora et al., 2022). The enzymes involved in this process include
312 phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), dihydroflavonol 4-reductase
313 (DFR), and leucoanthocyanidin reductase (LAR) (Deng and Lu, 2017). The activity and gene
314 encoding expression of these enzymes can be different among various plant species. For example,
315 higher activity of DFR and LAR in *P. prunelloides* could explain the increased proanthocyanidin
316 concentration. Comparisons of plant parts revealed that *Pentanisia prunelloides* and *Hypoxis*
317 *hemerocallidea* had higher proanthocyanidins in the roots than in the leaves. In contrast, *Merwillia*
318 *plumbea* and *Eucomis autumnalis* showed higher levels of proanthocyanidins in leaves than in
319 roots. The current findings were in agreement with Ncube et al. (2011) who found higher
320 concentrations of proanthocyanidins in *Hypoxis hemerocallidea* roots than in leaves whereas
321 *Merwillia plumbea* exhibited higher concentrations in leaves than roots. Specific transcription
322 factors such as myeloblastosis (MYB), basic helix-loop-helix (bHLH), and WD40 repeat (WD40)

323 which modulate flavonoid biosynthetic pathways might be differentially expressed in roots and
 324 leaves, resulting in the specific accumulation of proanthocyanidins in certain tissues (Pratyusha
 325 and Sarada, 2022).



326
 327 **Fig. 1.** The phytochemical concentration of different parts of *Merwillia plumbea*, *Pentanisia*
 328 *prunelloides*, *Hypoxis hemerocallidea*, and *Eucomis autumnalis*. The values are presented as
 329 means \pm SEM (n = 3) and LSD (n=24), the significant difference at the $p < 0.001$ level was

330 observed among various plant species and their plant parts (leaves and roots), means of treatments
331 sharing the same letter (s) are not significantly different at 5% level of significance. Abbreviations:
332 PS, plant species; PP, plant part. LSD, least significant difference.

333 **3.2 Antioxidant activity**

334

335 **3.2.1 Ferric reducing antioxidant potential assay and DPPH radical oxygen scavenger**

336

337 FRAP assay measures the capability of plant constituents to reduce ferric (Fe^{3+}) ions to ferrous
338 (Fe^{2+}) while DPPH assay quantifies the ability of compounds to scavenge free radicals by donating
339 hydrogen atoms (Bisht et al., 2024, Gulcin, 2020, Gulcin et al., 2019). The roots of *P. prunelloides*
340 showed a FRAP value of 2.97 mg/mL and a DPPH RSA quantified at 52.89%. In contrast, its
341 leaves demonstrated a FRAP of 2.60 mg/mL and a notable DPPH RSA of 35.25% (Table. 1).
342 Similarly, Mbhele (2021) found that *Pentanisia prunelloides* extracted with 50% methanol showed
343 good reducing activity of FRAP. *M. plumbea* roots had a FRAP value of 2.25 mg/mL and a higher
344 DPPH RSA of 80.86%, whereas the leaves revealed a FRAP of 1.66 mg/mL and a DPPH RSA of
345 66.25%. The antioxidant enzymatic systems such as superoxide dismutase, peroxidases, and
346 catalase may vary between roots and leaves, contributing to variations in the antioxidant potential
347 (Kasote et al., 2015). The roots and leaves of *H. hemerocallidea* recorded FRAP values of 1.85
348 and 1.93 mg/mL, respectively, with corresponding DPPH RSA values of 46.31% and 61.18%.
349 Both roots and leaves showed similar reducing power as reflected by FRAP values, however,
350 higher DPPH in leaves underscores their increased capacity to scavenge free radicals. This
351 variation may be an adaptive response to the changing environmental conditions faced by leaves
352 and roots. In this quest, leaves may require stronger protection against oxidative damage due to
353 their exposure to sunlight and atmosphere. *E. autumnalis* roots and leaves respectively exhibited
354 FRAP values of 2.78 mg/ml and 2.13 mg/ml with corresponding DPPH RSA of 80.72% and
355 74.54%. Elevated FRAP and DPPH in *E. autumnalis* roots indicated that roots possess strong
356 mechanisms to suppress oxidative stress due to highly effective antioxidant compounds or stress
357 hormones such as abscisic acid. However, this trend was not consistent across all plant species,
358 suggesting, that various species have different antioxidant profiles and activities, depending on
359 their specific compound's composition.

360 Table 1: The antioxidant activity [means \pm SEM (n = 3)] in various plant parts of *Merwillia*
 361 *plumbea*, *Pentanisia prunelloides*, *Hypoxis hemerocallidea*, and *Eucomis autumnalis*.

Plant Species	Plant Part	FRAP (mg.mL)	DPPH (RSA%)
		Mean \pm SE	Mean \pm SE
<i>P. prunelloides</i>	Roots	2.97 ^e \pm 0.37	52.89 ^c \pm 7.39
	Leaves	2.60 ^d \pm 0.23	35.25 ^a \pm 15.53
<i>M. plumbea</i>	Roots	2.25 ^c \pm 0.72	80.86 ^e \pm 4.27
	Leaves	1.66 ^a \pm 0.10	66.25 ^d \pm 8.24
<i>H. hemerocallidea</i>	Roots	1.85 ^{ab} \pm 0.10	46.31 ^b \pm 5.56
	Leaves	1.93 ^{abc} \pm 0.15	61.18 ^d \pm 6.07
<i>E. autumnalis</i>	Roots	2.78 ^{de} \pm 0.70	80.72 ^e \pm 0.71
	Leaves	2.13 ^{bc} \pm 0.40	74.54 ^e \pm 4.5
LSD		0.31	6.34
P-value		<.001	<.001

362 Abbreviations: FRAP, ferric reducing antioxidant potential assay; DPPH, 2, 2-diphenyl-1-
 363 picrylhydrazyl; \pm SE, minimum and maximum standard error; LSD, least significant difference.
 364 The values are presented as means (n = 3) and LSD (n=24), the significant difference at the $p <$
 365 0.001 level was observed among various plant species and their plant parts (leaves and roots),
 366 means of treatments sharing the same letter (s) are not significantly different at 5% level of
 367 significance.

368 3.3 Multivariate Analysis of Phytochemical Composition

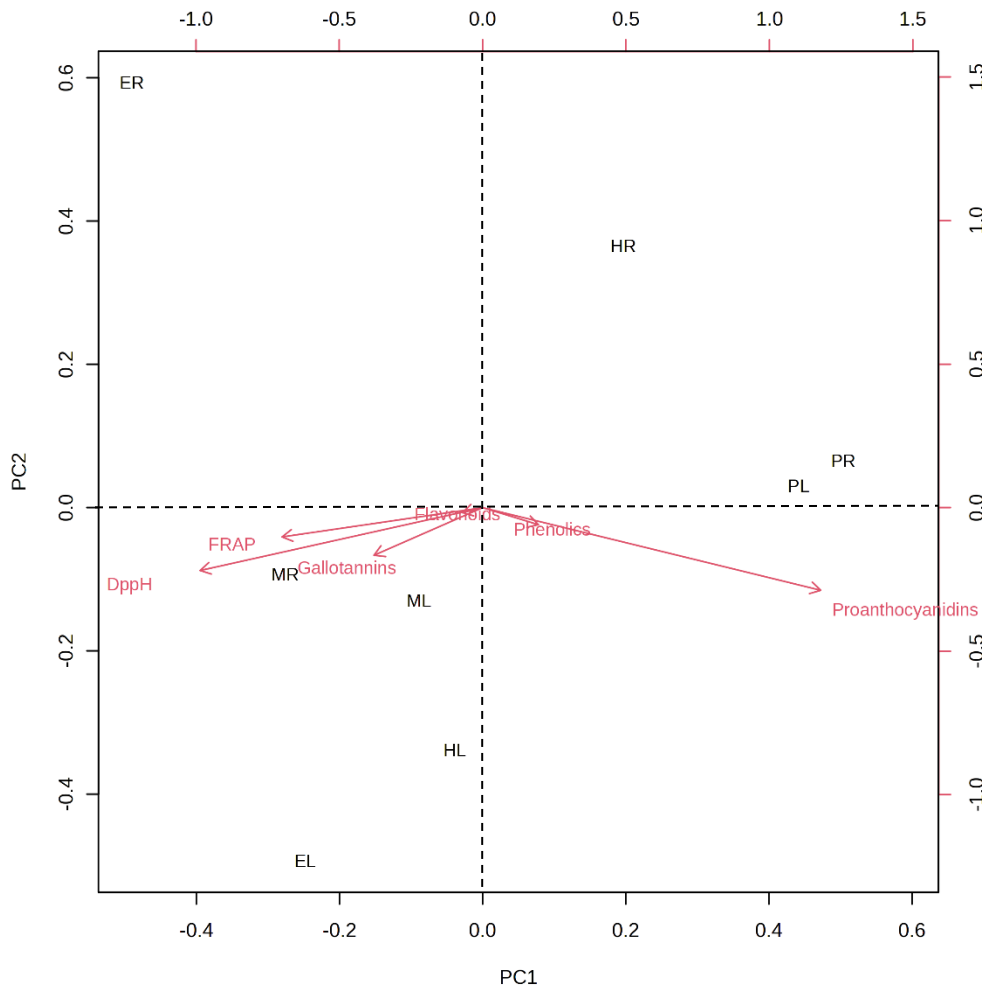
369

370 3.3.1 Principal components

371

372 The principal component analysis (PCA) was used to visualize the relationship between phenolics
 373 flavonoid, gallotannin, proanthocyanidins, FRAP, and DPPH under different plant species (Fig.
 374 2). PC1 explains the most variation in the data, with contributions from compounds that are aligned
 375 along the horizontal axis whereas PC2 present the next highest amount of variation, showing
 376 differences that are aligned along the vertical axis. The factor with higher loading scores means a
 377 tighter relationship with the same principal component. The PCA results revealed two significant

378 components PC1(90.1%) and PC2 (5.1%) that accumulated to 95.2 % of the total variance of the
 379 investigated parameters. The sample position showed *H. hemerocallidea* roots positively
 380 correlated with PC1 and PC2 indicating association with compounds that positively contribute to
 381 both components particularly, proanthocyanidins, phenolics, and flavonoids. *P. prunelloides* roots
 382 and leaves were positioned on the far right of PC1 suggesting a strong correlation to
 383 proanthocyanidins and phenolics. *E. autumnalis* roots located on the highest point of PC2
 384 indicating a strong association with compounds positively contributed to loading PC2. *M. plumbea*
 385 roots and leaves are positioned near the center and slightly on the negative score of PC1 and PC2
 386 representing alignment with gallotannins and moderate levels of flavonoids and phenolics. *E.*
 387 *autumnalis* leaves and *H. hemerocallidea* leaves are negatively associated with PC1 and PC2
 388 highlighting the alignment with FRAP and DPPH but lower compounds that define positive PC1
 389 and PC2.



390

391 **Fig. 2.** Principal component analysis chart for two principal components showing the relationship
392 between different plant parts of *Merwilla plumbea*, *Pentanisia prunelloides*, *Hypoxis*
393 *hemerocallidea*, and *Eucomis autumnalis*, and their compounds were analysed in triplicate.
394 Abbreviations: PCA, principal components; HL, *Hypoxis hemerocallidea* leaves; HR, *Hypoxis*
395 *hemerocallidea* roots; PL, *Pentanisia prunelloides* leaves; PR, *Pentanisia prunelloides* roots; EL,
396 *Eucomis autumnalis* leaves; ER, *Eucomis autumnalis* roots; ML, *Merwilla plumbea* leaves; MR,
397 *Merwilla plumbea* roots.

398 **3.4 LC-MS/MS identification of polyphenolic compounds**

399

400 **3.4.1 overview of identified compounds**

401

402 Based on the untargeted LCMS analysis, 1007 compounds were discovered across all four plant
403 species. Out of 1007 compounds, 776 were assigned names while 231 remained unclassified.
404 There were 27 major compounds identified from different plant extracts that showed potential to
405 be used as anti-obesity and anti-inflammatory (Table 2). The selection criteria were based on the
406 literature reported on the biological activities of compounds including, their ability to inhibit lipid
407 metabolic enzymes and inflammatory pathways. These compounds were classified into 6
408 categories including phenolic acids, flavonoids, terpenoids, saponins, fatty acids, and other
409 polyphenols.

410

411 **3.4.2 Phenolic acids**

412 The three phenolic acids observed were tentatively characterized through MS/MS fragmentation.
413 The compound gentesic acid 5-O-glucoside (m/z 315.07) exhibited daughter ions at m/z 153 and
414 m/z 109 by the loss of the glucoside moiety (162 Da) and carboxyl (44 Da), respectively (Mashitoo
415 et al., 2021). Similarly, Karageçili et al. (2023a), Karageçili et al. (2023b) reported daughter ions
416 at m/z 152.8 and m/z 109 for gentesic acid. This compound reduces fat accumulation in adipose
417 tissues by inhibiting key enzymes such as α -amylase and α -glucosidase involved in carbohydrate
418 digestion. Caffeic acid 4-O-glucuronide (m/z 355.07) produced ions of m/z 193 and m/z 123,
419 corresponding to loss of carboxyl group and glucuronide moiety. Chlorogenic acid (m/z 353.09)
420 showed the fragment ions of m/z 191 correspond to the caffeic acid moiety after the loss of quinic
421 acid and m/z 135 represents the loss of quinic acid moiety as previously described by Yunus et al.

422 (2021). Similarly, Karageçili et al. (2023a), Ozden et al. (2023) found Chlorogenic acid at m/z
423 353. Chlorogenic acid is an ester caffeic acid and quinic acid known to inhibit glucose absorption
424 and improve insulin sensitivity, however, the replacement of quinic acid with glucuronic acid can
425 produce chlorogenic acid mimics with increased antioxidant activity (Cao et al., 2019).

426 3.4.2 Flavonoids

427 Eight flavonoids were identified in the selected plant extracts and their structures were confirmed
428 by fragment patterns. Prodelphiniline was identified at m/z 609.13, producing fragment ions at m/z
429 175 which represents a huge loss of major structural components and at m/z 333 indicating the loss
430 of sugar moieties (Ancillotti et al., 2017). Procyanidin C1 exhibited m/z 865.2 which formed
431 fragment ions at m/z 287 and m/z 577 representing catechin unit and partial loss of dimetric
432 structure respectively (Zhang et al., 2023). Myricitrin I (m/z 477.14) showed daughter ions of
433 m/z 109 which indicated a major loss of sugar moiety and m/z 175 suggested further loss in
434 flavonoid core structure. Apigenin 7-O-(6"-O-acetylglucoside) was detected at m/z 473.11 and
435 showed fragment ions of m/z 175 and m/z 239 by the loss of acetyl or glucose group and additional
436 fragmentation of flavonoid core structure respectively. Tricin 7-Glucoside (m/z 491.12) exhibited
437 daughter ions of m/z 109 and 161 representing a loss of glucose moiety and flavonoid glycosides
438 respectively. The compound malvidin 3-rutinoside (m/z 635.20) produced fragment ions at m/z
439 133 which represented the loss of glycosidic bonds of flavonoids and m/z 279 indicating additional
440 loss within the anthocyanin structure (Ling et al., 2009). Quercetin 3-O-alpha-(6"-
441 Caffeoylglucosyl-beta-1,2-Rhamnoside) identified at m/z 771.18 which produced daughter ions
442 m/z 255 and m/z 609 by the loss of rhamnoside or caffeoyl groups as previously described by Li
443 et al. (2021). Quercitrin (m/z 447.09) exhibited fragmentation at m/z 146, indicating a major loss
444 of glycosidic bond, and at m/z 255, confirming a core flavonoid structure. Quercitrin is the most
445 abundant form of a glycoside of quercetin, quercetin O-glycoside is that quercetin substituted at
446 position 3 via a glycosidic bond to the alpha-L-rhamnosyl moiety (De Paula Alves et al., 2022).
447 These flavonoids exhibit antioxidant activities, anti-obesity, and anti-inflammatory properties by
448 regulating lipid metabolism, decreasing adipogenesis through reducing the expression of PPAR γ
449 and C/EBP α , and pro-inflammatory cytokines (Toma et al., 2020). Additionally, procyanidin C1,
450 prodelphiniline, and malvidin 3-rutinoside decrease the level of reactive oxygen species by
451 upregulating superoxide dismutase (SOD) in endothelial cells (Merecz-Sadowska et al., 2023).

452 3.4.3 Terpenoids/Triterpenoids

453
454 Terpenoids are bioactive compounds known for their pharmacological activities and therapeutic
455 effects in regulating lipid metabolism and reducing oxidative stress (Yao and Liu, 2022). Geranyl
456 arabinopyranosyl-glucoside (m/z 447.23) showed fragmented ions of m/z 113 suggesting the
457 removal of a larger portion of a molecule, potentially associated with core sugar unit and
458 hydrocarbon chain, and m/z 293 indicated a further loss of the glucoside moiety (Stranska et al.,
459 2021). This compound has been documented to increase insulin sensitivity by inhibiting protein
460 tyrosine phosphatase 1B (PTP1B) (Genovese et al., 2021). The compound lancemaside G (m/z
461 1283.59), triterpene saponins (m/z 1283.59), scillascolloside E2 (m/z 1269.58) and glucosyl
462 (2E,6E,10x)-10,11-Dihydroxy-2,6-Farnesadienoate (m/z 431.23) exhibited daughter ion at m/z
463 187, m/z 113, m/z 113 and 147 respectively which indicated a loss of larger portion from the core
464 triterpene structure (Raheem et al., 2019). The observed fragmentation pattern of these compounds
465 corroborates the structural identities of triterpene saponins which are recognized for their anti-
466 obesity and anti-inflammatory properties.

467 3.4.4 Fatty Acids

468 Fatty acids are classified as carboxylic acids with aliphatic tails of different lengths, for example,
469 carboxylic acids with aliphatic tails of less than 6 carbons are referred to as short-chain fatty acids
470 (Layden et al., 2013). The current results exhibited the long chain fatty acids including,
471 corchorifatty acid F (m/z 327.22), 9,12,13-triHOME (m/z 329.23) and gallicynoic acid F (m/z
472 343.21) at similar fragments of m/z 146 which suggested common structural element, potential
473 associated with fatty acid's hydrocarbon chain and m/z 174 indicated the loss of carbon dioxide.
474 These compounds play an essential role in lipid metabolism and reduce the production of
475 proinflammatory cytokines such as TNF- α and IL-6 (Vasan et al., 2019, Zhang et al., 2024).
476 (9S,10S) -9,10-Dihydroxyoctadecanoate (m/z 315.25) produced daughter ions at m/z 174 and m/z
477 243 respectively representing the breakdown of carbon bonds and loss of the major alkyl group
478 (Burdge and Calder, 2015). This compound is known to be linked with insulin sensitivity through
479 the activation of the AMP-activated protein kinase (AMPK) pathway to promote glucose uptake
480 by the muscle and fatty acid oxidation (Ke et al., 2018).

481 3.4.5 Saponins

482 Beta-D-glucose pentaacetate (m/z 389.1) generated daughter ions at m/z 141 through the loss of
483 acetylated glucose moiety. The presence of acetylated glucose in the structure of this compound
484 may influence glucose metabolism by regulating α -amylase and α -glucosidase (Wang et al., 2018).
485 The compound beta-d-xylopyranosyl-(1->2) (m/z 1163.55) showed fragmentation at m/z 101 and
486 m/z 149 with the loss of xylose backbone. The structural fragmentation is significant because
487 xylose may inhibit the hydrolysis of glycosidic bonds by digestive enzymes in the small intestine,
488 thereby reaching the colon intact to be fermented by beneficial anaerobic bacteria (Arjona et al.,
489 2007). The fermentation process promotes gut microbiota which manages metabolic disorders and
490 regulation of inflammation. Trigoneoside XIIIa identified at m/z 1271.59 exhibited fragment ions
491 at m/z 293 representing the loss of sugar fragments which confirmed complex glycosidic
492 structures, particularly of saponins (Kang et al., 2013). This compound reduces pro-inflammatory
493 cytokines such as TNF- α and IL6 by regulating the activities of NFkB.

494 3.4.6 Other polyphenolic compounds

495
496 Broussonetine F (m/z 360.24) exhibited product ions at m/z 133 indicating the minimal loss of
497 alkaloids and at m/z 281 confirming the core alkaloid structure (Laili et al., 2023). This compound
498 suppressed inflammation in RAW264.7 cells by inhibiting extracellular signal-regulated kinase
499 (ERK) and p38 mitogen-activated protein kinase (p38 MAPK) (Huang et al., 2019). Daucic Acid
500 (m/z 203.02) exhibited a fragment ion identical to the parent ion suggesting a stable structure. The
501 compound mycophenolic acid O-acyl-glucuronide (m/z 495.15) produced daughter ions at m/z 151
502 and m/z 297, indicating loss of glucuronide moiety. Mycophenolic acid is commonly used as
503 an immunosuppressant, preventing organ rejection after transplanting by inhibiting the enzyme
504 inosine monophosphate dehydrogenase (IMPDH) that promotes the proliferation of T-cell and B-
505 cell (Shigesaka et al., 2020). Suppressing the immune cell activity may reduce inflammatory
506 response and improve insulin sensitivity and metabolic health. D-Linalool 3-Glucoside (m/z
507 315.18) exhibited fragment ion at m/z 146 representing the loss of a small group of potential
508 glucoside or linalool moiety. The fragmentation of glucose moiety may increase the bio-
509 availability of linalool, suggesting its potential to mitigate the activities of NF-kB and MAPK and
510 improve insulin sensitivity (Sabogal-Guáqueta et al., 2018).

511 Table 2. Based on the LCMS analysis, twenty-seven compounds were discovered from different plant parts of *P. prunelloides*, *E.*
 512 *autumnalis*, *M. plumbea*, and *H. hemerocallidea* to have the potential to prevent or treat obesity-induced inflammation. All analyses
 513 were conducted in triplicates.

Compound	Formula	Average Rt(min)	Ionization ESI (+/-)	Molecular weight (g/mol)	Average M/z	Plant part
Phenolic Acids						
Gentesic acid 5-O-glucoside	C13H16O9	3.4	[M-H] ⁻	316.26	315.07	HL, HR, PL, PR
Caffeic acid 4-O-glucuronide	C15H16O10	3.7	[M-H] ⁻	356.28	355.07	HL, EL, PL
Chlorogenic acid	C16H18O9	4.0	[M-H] ⁻	354.31	353.09	HL, HR, PL, ML, MR
Flavonoids						
Prodelpiniline	C30H26O14	3.0	[M-H] ⁻	610.50	609.13	PL, PR
Procyanidin C1	C45H38O18	3.2	[M-H] ⁻	866.80	865.20	PL, PR
Myricitrin I	C23H26O11	4.1	[M-H] ⁻	478.45	477.14	HL, HR, ML, MR
Apigenin 7-O-(6"-O-acetylglucoside)	C23H22O11	4.3	[M-H] ⁻	474.40	473.11	HR, HL, EL, ML
Tricin 7-glucoside	C23H24O12	4.3	[M-H] ⁻	492.40	491.12	HL, HR
Malvidin 3-rutinoside	C30H36O15	5.1	[M-H] ⁻	636.60	635.20	HR, HL, EL, ER, ML, MR
quercetin caffeoylglucosyl-beta-1,2-rhamnoside)	3-O-alpha-(6'- C36H36O19	5.2	[M-H] ⁻	772.70	771.18	HL, HR, ML, MR
Quercitrin	C21H20O11	5.2	[M-H] ⁻	448.40	447.09	HR, EL, PL
Terpenoids/Triterpenoids						

Geranyl arabinopyranosyl-glucoside	C21H36O10	6.5	[M-H] ⁺	448.50	493.23	HL, HL, ER, ML, MR
Lancemaside G	C57H90O27	7.5	[M-H] ⁻	1207.3	1205.56	EL, ER, ML, MR
Triterpene saponins (UNPD166363)	C59H96O30	7.3	[M-H] ⁺	1283.59	1283.59	EL, ER, ML, MR
Scillascilloside E2	C57H92O28	7.6	[M-H] ⁺	1225.30	1269.58	HL, EL, ER, ML, MR
Glucosyl (2E,6E,10x)-10,11-dihydroxy- 2,6-farnesadienoate	C21H36O9	7.5	[M-H] ⁻	432.50	431.23	HL, HR, EL, ER, PL, PR, ML, MR
Fatty Acids						
Corchorifatty acid F	C18H32O5	7.0	[M-H] ⁻	328.44	327.22	HL, HR, EL, ER, PL, PR, ML, MR
9,12,13-TriHOME	C18H34O5	7.4	[M-H] ⁻	330.50	329.23	HL, HR, EL, ER, PL, PR, ML, MR
(9S,10S)-9,10-dihydroxyoctadecanoate	C18H36O4	9.7	[M-H] ⁻	315.50	315.25	HL, HR, EL, ER, PL, PR, ML, MR
Gallicynoic acid F;(-)-Gallicynoic acid F	C18H32O6	9.0	[M-H] ⁻	344.40	343.21	HL, HR, EL, ER, PL, PR, ML, MR
Saponins						
Beta-D-Glucose pentaacetate	C16H22O11	3.1	[M-H] ⁻	390.34	389.11	PL, PR
BETA-D-XYLOPYRANOSYL-(1->2)	C55H88O26	7.1	[M-H] ⁻	1165.30	1163.55	EL, ER
Trigoneoside XIIIa	C57H94O28	7.9	[M-H] ⁻	1227.3	1271.59	HL, EL, ER, ML, MR
Other polyphenols						
Broussonetine F	C18H35NO6	5.8	[M-H] ⁻	361.5	360.24	HL, HR, EL, ER, PL, PR, ML, MR

Daucic acid	C7H8O7	2.2	[M-H] ⁻	204.13	203.02	HL, HR, PL
Mycophenolic acid O-acyl-glucuronide	C23H28O12	3.9	[M-H] ⁻	496.50	495.15	HL, HR, ML, MR
D-Linalool 3-glucoside	C16H28O6	8.0	[M-H] ⁻	316.39	315.18	HL, HR, EL, ER, PL, PR, ML, MR

514 Abbreviations: HL, *Hypoxis hemerocallidea* leaves; HR, *Hypoxis hemerocallidea* roots; PL, *Pentanisia prunelloides* leaves; PR,
515 *Pentanisia prunelloides* roots; EL, *Eucomis autumnalis* leaves; ER, *Eucomis autumnalis* roots; ML, *Merwillia plumbea* leaves; MR,
516 *Merwillia plumbea* roots; M-H, deprotonated molecular ion; M/z, mass-to-charge ratio; Rt, retention time; ESI, electrospray ionization.

517 **4. Conclusion**

518 The study successfully identified and characterised polyphenolic compounds in *P. prunelloides*,
519 *E. autumnalis*, *M. plumbea*, and *H. hemerocallidea* using LC-MS/MS and quantified their
520 concentrations via UV spectrophotometry. The results revealed significant variation in phenolic
521 acids, flavonoids, terpenoids, saponins, fatty acids, and other polyphenols among plant species and
522 parts with *P. prunelloides* exhibiting the highest concentrations. These variations in antioxidant
523 potential and metabolite distribution support the hypothesis that specific plant species and parts
524 accumulate different levels of secondary metabolites and are influenced by genetic expression,
525 enzymatic activity, and environmental stress adaptation.

526 These findings have significant implications for medicinal and pharmaceutical applications, as the
527 identified polyphenols exhibit potential in lipid metabolism regulation, oxidative stress protection,
528 and anti-inflammatory activity. However, considering sustainability, it is preferable to harvest
529 leaves instead of roots if they provide an adequate compound concentration to ensure long-term
530 conservation. Among the plant species analysed, *P. prunelloides* exhibited the highest metabolite
531 concentration, making it a strong candidate for further research into its therapeutic applications.
532 Future research should validate the bioactivity of its key compounds in vivo, explore seasonal
533 variations, and assess optimal harvesting practices for sustainable utilization of these medicinal
534 plants.

535

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538

539 **5. References**

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