

Phytochemical Profiling and Nutritional Assessment of Indigenous Cowpea (*Vigna unguiculata* L.) Cultivars from Bulukumba, South Sulawesi, Indonesia

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Abstract

Cowpea (*Vigna unguiculata* L.) is a leguminous crop recognized for its high nutrient density and substantial potential as a functional food. Bulukumba Regency in South Sulawesi, Indonesia, harbors two locally adapted red and white cultivars that have been traditionally cultivated and consumed as staple foods. This study aimed to evaluate their nutritional composition, antioxidant capacity, and bioactive compound profiles. Proximate composition was determined following the AOAC protocols, tannin content was quantified using a modified vanillin-HCl assay, antioxidant activity was assessed via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and bioactive compounds were identified through Gas Chromatography–Mass Spectrometry (GC–MS). Both cultivars exhibited high protein (20.47–21.03%) and carbohydrate (43.93–51.91%) contents, low lipid content (0.63%), and comparable ash content (3.25–3.28%). Tannin content was substantially higher in the red cowpea (465.61 µg/g) than in the white cowpea (130.43 µg/g). Antioxidant activity was significantly greater in the red cultivar (2,115.33 µg/g) compared with the white cultivar (507.18 µg/g). GC–MS analysis revealed a diverse range of bioactive compounds, including fatty acids (oleic, palmitic, and pentadecanoic acids), alcohols, esters, nitrogen-containing molecules, vitamins, terpenoids, and phenolics—many of which are reported to possess antioxidant, antimicrobial, anti-inflammatory, anticancer, and cardioprotective properties. These findings highlight the superior functional potential of the red cultivar and support the valorization of local cowpea germplasm for improved nutrition, human health, and sustainable agricultural development.

Keywords: Antioxidant, bioactive compounds, proximate composition, *Vigna unguiculata*

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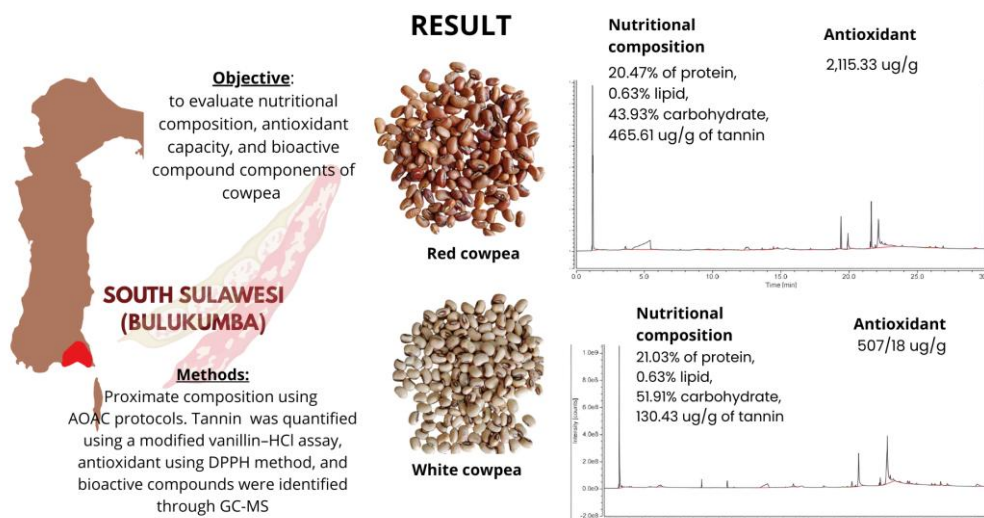
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Graphical Abstract



Introduction

Legumes are nutrient-dense, plant-based foods that contain a variety of bioactive compounds with potential health benefits. Among them, cowpea (*Vigna unguiculata*) is a particularly promising local legume. Belonging to the Fabaceae family, cowpea is an annual leguminous crop known for its high adaptability to poor soil conditions and suboptimal climates, as well as its relatively short growing cycle [1,2,3]. This species is widely cultivated across tropical regions, including South Sulawesi, particularly in Bulukumba Regency. Although cowpeas have been traditionally grown and consumed by local communities in Bulukumba for generations, the nutritional composition and bioactive properties of these red and white varieties remain scientifically underexplored.

Previous studies have identified cowpeas as important grain legumes that serve as versatile sources of food and high-quality protein. The nutritional profile of a food ingredient typically includes its proximate composition, comprising moisture, ash, protein, fat, and carbohydrate content [4]. Investigations into the nutritional

composition of cowpeas have been conducted in various countries. Cowpeas generally contain approximately 50–60% carbohydrates and 18–35% essential proteins [5,6]. In addition, they exhibit low fat content (around 1.5%) and are rich in essential vitamins and minerals, including calcium (Ca), phosphorus (P), iron (Fe), folate, thiamine, and riboflavin [7].

Moreover, several secondary metabolites such as phenols, sterols, and flavonoids have been identified in cowpea seeds [8]. The presence of both primary and secondary metabolites indicates that cowpeas possess potential antioxidant, anticancer, and antibacterial properties, attributed to their peptide, lipid, phenolic, and sterol content [9][10].

The difference in seed coat color cowpea cultivars hypothesized to influence the content of anthocyanins and polyphenols, compounds that contribute to antioxidant activity [11]. Anthocyanins are flavonoid compounds that function as pigments and possess antioxidant properties [12]. Dark colored seed coats in legumes are associated with higher phenolic compound content compared to light colored varieties

[13]. Therefore, a comparative study of these several cultivars essential for identifying the nutritional potential and bioactive compound content.

In natural environments, plants are exposed to various abiotic stresses that can adversely impact growth, productivity, and seed yield [14]. Water availability plays a critical role in nutrient absorption and translocation; under water-limited conditions, nutrient uptake is impaired. Such stress can inhibit cell division and elongation, reduce leaf area, limit root and stem development, and disrupt stomatal conductance [15]. Severe soil moisture deficits have also been associated with reductions in protein content and shifts in nutrient composition. In addition to environmental factors, the chemical and nutritional characteristics of cowpea seeds are significantly influenced by genetic variation, particularly differences between cultivars [16][17].

The collection of local accessions in South Sulawesi exhibits genetic diversity that enables the selection of superior genotypes (e.g., early maturity and stress tolerance). Consequently, the Bulukumba accession has the potential to possess better agronomic traits and environmental adaptability. The agroecological conditions in the Bulukumba region may influence the nutritional content and bioactive components of cowpea seeds, leading to differences in the chemical composition compared to other regions. Furthermore, the tropical agroclimatic conditions, characterized by a dry season in Bulukumba, support the adaptation of drought-tolerant cowpea varieties. This allows local varieties to maintain stable yields on marginal lands, providing agronomic advantages for local farmers [18].

This study aims to analyze the nutritional composition, evaluate the antioxidant activity, and identify the bioactive

components of cowpea (*Vigna unguiculata*) cultivars originating from Bulukumba Regency, South Sulawesi, Indonesia. The nutritional composition will be determined through proximate analysis, antioxidant activity will be evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and bioactive compounds will be identified via Gas Chromatography–Mass Spectrometry (GC–MS). The findings are expected to provide scientific evidence supporting the utilization of cowpeas as a nutritious and functional food, while promoting the valorization of local commodities and fostering new market opportunities for the regional agricultural sector.

Materials and Methods

Materials

Fresh cowpea (*Vigna unguiculata* L.) seeds were obtained from local agricultural markets in Bulukumba Regency, and manually sorted to ensure uniformity. All reagents were of analytical grade. Absolute methanol was used for extraction, vanillin–HCl reagent and catechin standard were employed for tannin analysis, DPPH reagent (Merck, Germany) and HPLC-grade methanol, silica gel (60–120 mesh), dichloromethane, and ultra-high-purity helium gas were used for GC–MS analysis. Instrumentation included a UV–visible spectrophotometer, rotary evaporator, centrifuge, and a GC–MS–QP 2010 SE system equipped with a Restek capillary column.

Sample preparation

Whole red and white cowpea seeds were manually sorted to eliminate foreign materials and debris. After thorough cleaning, the seeds were weighed and stored in sealed, transparent low-density polyethylene bags at 4°C until further analysis. Before use, the cowpea seeds were processed through a 500 µm sieve using a

screening machine to create uniform whole grain flour.

Proximate analysis

Proximate analysis. The AOAC (2006) procedure was used to determine the proximate composition of the prepared red and white cowpea flour samples. Proximate parameters were moisture content, ash, protein, lipid, and carbohydrate [4,19,20].

Tannin analysis

Quantitative analysis of tannin compounds using a modified vanillin-HCl procedure. Each flour sample (2 gram) from both red and white cowpeas was extracted using absolute methanol with continuous agitation for 20 min at room temperature. The extract was then centrifuged for 10 min, and 1 mL of the supernatant was reacted with 5 mL of vanillin-HCl reagent then incubated for 20 min at room temperature. After incubation, the absorbance of the formed complex was measured at wavelength of 500 nm. Quantification was performed based on a catechin calibration curve with a blank correction, and the results were expressed as mg of catechin equivalent [21].

Antioxidant analysis

The antioxidant capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method, following the protocol described by Sadh et al. [22] with minor modifications. A stock solution containing 0.1 mM DPPH reagent (Merck, Germany) was prepared by dissolving 4 mg DPPH in 100 mL analytical grade methanol. Acetonic extracts (200 μ L) were combined with 2 mL of the DPPH working solution and incubated in darkness for 30 min at room temperature. Absorbance measurements were recorded at 517 nm against a methanol blank.

GC-MS analysis

A total of 50 g of each cowpea flour sample is extracted using methanol solvent with a ratio of 1:4. The mixture is extracted for 48 h. The extraction results are filtered using filter paper and concentrated using a rotary evaporator at a temperature of 40 °C. The extracts were concentrated in vacuum using rotary evaporator at a temperature of 50 °C, then partially purified by adsorbing to silica and eluting with 20 mL of dichloromethane in a column. The eluates obtained were concentrated, reduced to 2 mL and analyzed using GC-MS. Identification of the compounds in cowpeas was carried out using method described by Odion & Usifoh [23]. It involves using a GC-MS-QP 2010 SE Shimadzu, (JAPAN). The column used was a Restek column with length, internal diameter and thickness (30 m x 0.32 mm x 0.5 μ m) with the following conditions: the GC was operated in the splitless injection mode with 1 mL/min flow rate for helium gas as carrier gas and make up gas. The injection temperature and volume are 250 °C and 8 μ L respectively. Samples were injected automatically by split-less mode into the MS at an interface temperature of 250 °C with ion source at 230 °C, with ionization mode of electron impact ionization (EI) of 70 eV. Three ions specifically; the most abundant as quantification ion and two ions for confirmation.

Result and Discussion

This study utilized local cowpea varieties from Bulukumba Regency that exhibited significant variation in seed color. Two cultivars were identified, characterized by reniform or kidney shaped seeds with smooth seed coat surface and distinct color variants comprising red and white seed (Figure 1).



Figure 1. Cowpea seed coat pattern, (a) Red, (b) White.

Nutrition composition

The nutritional composition analysis, comprising proximate parameters and

tannin content, was conducted on red and white cowpeas collected from Bulukumba Regency, South Sulawesi. The results are summarized in Table 1.

Table 1. Nutritional composition data: moisture, ash, protein, fat, carbohydrate, and tannin content for red and white cowpea cultivars

Parameter content	Red cowpea	White cowpea
Ash (%)	03.25	03.28
Moisture (%)	09.44	08.30
Protein (%)	20.47	21.03
Lipid (%)	0,04375	0,04375
Carbohydrat (%)	43.93	51.91
Tannin (µg/g)	465.61	130.43.00

As shown in Table 1, nutritional composition of red and white cowpeas shows no significant differences. For instance, the ash content of red cowpea is 3.25%, while that of white cowpea is 3.28%. Ash content represents the inorganic residue remaining after the removal of moisture and organic matter through incineration, and serves as an indicator of the total mineral content in a sample [23]. These values suggest that both cowpea cultivars contain comparable levels of minerals. Interestingly, the ash content of the local cowpeas from Bulukumba is similar to that of local peanuts from Southwest Maluku, which ranges from 3.13% to 3.97% [4]. Conversely, it differs from the ash

content of Nigerian cowpeas, reported at a lower value of 2.78% [23]. These findings indicate that mineral content in legumes may vary across geographical regions and can differ substantially among cultivars due to environmental and physiological factors.

The moisture content analysis showed that the red cowpea had a slightly higher moisture level (9.44%) compared to the white cowpea (8.30%). This value is lower than the moisture content of cowpea utilized in Nigeria, which is reported to be 13.0% [19]. Moisture content refers to the amount of water present in a material or food product. Measuring moisture content is crucial for

determining shelf life and for managing post-harvest handling, processing, and distribution [24]. The moisture values obtained indicate that both cowpea cultivars exhibit a relatively low moisture level, below 10%, which suggests good dryness and the potential for extended shelf life when stored under appropriate conditions [25].

The protein content of white cowpea was slightly higher at 21.03%, compared to 20.47% in red cowpea. These values are notably higher than those reported for cowpeas commonly consumed in the Sahelo-Saharan zone of Chad, which range from 18.89% to 19.41% [26]. The relatively high protein content of the local cowpeas from Bulukumba supports their potential utilization as a valuable source of plant-based protein. This finding is consistent with Boukar et al. [3], who reported that cowpea protein content generally falls within the range of 20–25%.

Similarly, the white cowpea exhibited a higher carbohydrate content (51.91%) than the red variety (43.93%). The high carbohydrate level contributes significantly to the overall energy value, suggesting that the white cultivar could serve as a good source of plant-derived energy. When compared to other regions, the carbohydrate content of the Bulukumba variety is considerably lower than that of cowpea varieties from Swaziland, which range between 45.64–57.12% [27], and those from Maluku, which range between 58.46–63.48% [4]. Both cowpea cultivars showed identical fat content, measured at 0.63%. This value is relatively low, which aligns with the general characteristic of legumes that are not primary sources of fat, when compared to the fat content of cowpea from Mpumalanga, South Africa, which is higher at 2.65% [20]. The low fat content makes cowpea a suitable dietary option for individuals adhering to low-fat diets [28].

The nutritional composition of the Bulukumba cowpea variety differs from that of cowpea grown in other regions such as Maluku, Nigeria, Swaziland, Mpumalanga, and the Sahelo-Saharan Zone of Chad. These regional variations are influenced by several factors, including genetic factors (plant varieties) [29], and environmental conditions (soil and climate), where temperature and water availability affect plant metabolism—thus periods of drought or extreme heat can alter seed composition [30]. Cultivation practices, such as fertilizer application (type and dosage), crop rotation, planting density, and irrigation, also affect nutrient availability and plant stress, which in turn influence seed composition [31]. Post-harvest handling, particularly at different maturity stages, results in varying levels of moisture, protein, and carbohydrates [32].

Each variety possesses distinct physiological abilities to absorb nutrients from the soil and regulate metabolism during growth; therefore, its nutritional expression depends on the growing environment [33]. Moreover, the interaction between genotype and environment strongly determines the variation in protein, mineral, and bioactive compound contents. Factors such as soil fertility, pH, rainfall, temperature, and water availability directly influence photosynthetic efficiency and the formation of nutritional compounds in the seeds [34].

Tannin analysis.

Tannins are phenolic compounds that act as antinutrients; however, in recent years, they have been shown to possess various health-promoting properties, including antioxidant, anticarcinogenic, antimutagenic, and antimicrobial activities [35]. The red cowpea contained a tannin concentration of 465.61 µg/g, which is substantially higher than that of the white cowpea, measured at 130.43 µg/g. The higher tannin content in red

cowpea may contribute to enhanced antioxidant activity, whereas the lower tannin content in white cowpea could potentially result in improved nutrient bioavailability. Pigmented cowpea seeds have been associated with higher levels of tannins, total phenolics, total flavonoids, ferric reducing ability, and lipid peroxidation inhibition compared to non-pigmented seeds [36].

Antioxidant activity

Antioxidant activity refers to the overall capacity of a substance to scavenge free radicals within cells [37]. In this study, the antioxidant activity of red and white cowpeas was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, a widely used method known for its simplicity, speed, and accuracy. DPPH is a stable free radical characterized by an unpaired electron on a nitrogen atom. When a hydrogen-donating antioxidant compound reacts with DPPH, it reduces the DPPH radical to DPPH-H (1,1-diphenyl-2-picrylhydrazine) [38].

The antioxidant activity of red cowpea was recorded at 2,115.33 $\mu\text{g/g}$, substantially higher than that of white cowpea, which measured 507.18 $\mu\text{g/g}$. This pronounced difference suggests that the red cowpea cultivar possesses significantly stronger antioxidant potential compared to the white cultivar. The difference in antioxidant activity between red and white cowpea cultivars corresponds to the variation in their tannin content. The red cultivar, which contains higher levels of tannins, exhibits a significantly greater DPPH radical scavenging capacity compared to the white cultivar. This indicates that tannins play an important role as phenolic compounds contributing to antioxidant capacity through the mechanism of hydrogen or electron donation to free radicals [39]. This finding is consistent with previous studies showing

that pigmented legume seeds possess higher tannin and total phenolic contents as well as stronger antioxidant activity than non-pigmented varieties [40]. Therefore, the higher tannin content in the red cultivar can be considered a key factor enhancing antioxidant activity, while the lower tannin level in the white cultivar may provide advantages in terms of nutrient bioavailability. The elevated tannin content and antioxidant activity in red cowpea reinforce the role of pigmented cultivars as potential sources of bioactive compounds beneficial to human health [41]. High antioxidant activity is crucial for neutralizing free radicals in the body, thereby helping to prevent oxidative stress, a major contributor to various degenerative diseases such as cancer, cardiovascular disorders, and diabetes [42].

Identification of bioactive compounds

The results of the antioxidant activity assay, which demonstrated a strong capacity to scavenge free radicals such as DPPH, indicate the presence of antioxidant bioactive compounds in cowpea seeds. To further identify the bioactive constituent presented in both red and white cowpea cultivars from Bulukumba, Gas Chromatography–Mass Spectrometry (GC-MS) analysis was conducted. GC-MS is widely recognized for its rapid analysis and ability to identify unknown compounds in complex mixtures.

The GC-MS analysis of methanolic extracts from red and white cowpeas revealed the presence of various bioactive compound derivatives. Based on chromatogram interpretation and library matching, each cultivar was found to contain several compounds with potential biological activity. Figure 2 presents the chromatogram of the red cowpea extract, showing distinct peaks at various retention times.

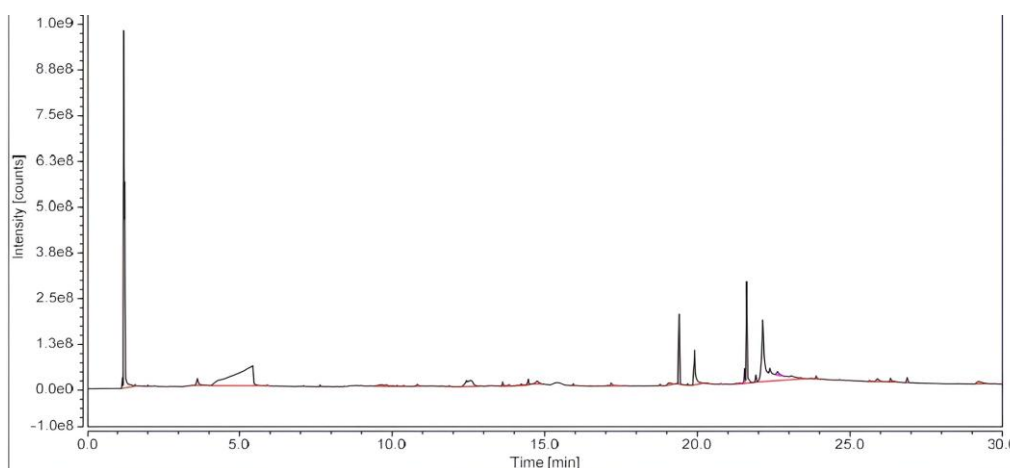


Figure 2. GC-MS chromatogram of red cowpea (*Vigna unguiculata*) extract.

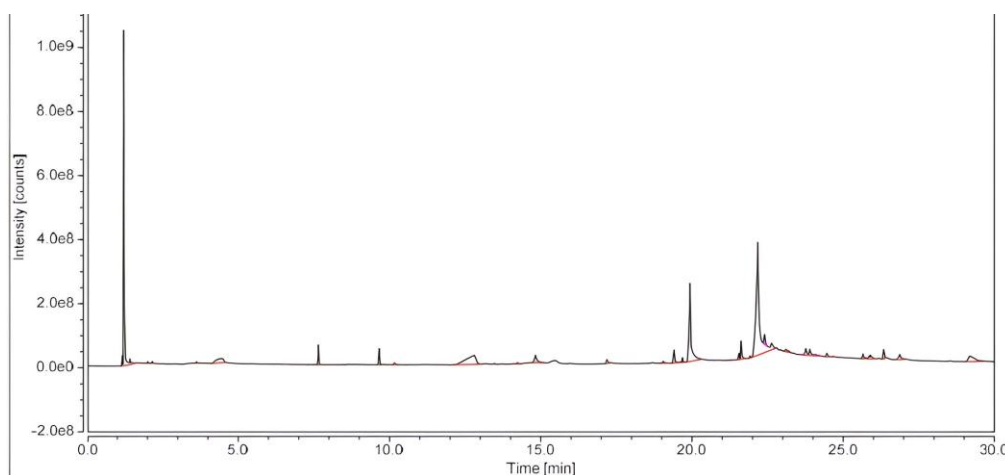


Figure 3. GC-MS chromatogram profile of white cowpea methanol extract showing major compound peaks

Based on the chromatogram shown in Figure 2, a total of 80 distinct peaks were detected, indicating the presence of various bioactive compounds. The bioactive compounds identified through GC-MS analysis of the methanolic extract of red cowpea are presented in **Supplementary Table S1**. Methanolic extract of red cowpea contained several compounds with significant relative abundance. The highest relative area was observed at peak 2, accounting for 17.22% of the total area, with a retention time of 1.204 minutes. This peak corresponds to three possible compounds: hydroxylamine, 1,2-ethanediol, and difluoromethane.

Another major peak was observed at peak 12, representing 8.94% of the total relative area. The compounds identified at this peak include glycerin, erythritol, and glyceraldehyde. Additionally, peak 63 showed a relative abundance of 7.08%, corresponding to trans-13-octadecenoic acid methyl ester, oleic acid, and cis-vaccenic acid.

For better comparative understanding of the chemical profiles, GC-MS analysis was also performed on the methanolic extract of the white cowpea cultivar. The GC-MS chromatogram of the methanolic extract of white cowpea revealed 60 distinct peaks (Figure 3), indicating the presence of various chemical constituents. The identified

compounds corresponding to these peaks are listed in **Supplementary Table S2**.

GC-MS analysis of white cowpea methanol extract revealed that the compound with the highest relative area of 23.25% was detected at peak 2 with a retention time of 1.204 min. Three potential compounds were identified at this peak: hydroxylamine, 1,2-ethanediol, and hydroxyurea. These compounds are consistent with those found in red cowpea at the same retention time, although the relative peak area in red cowpea was lower. At peak 32 (retention time 19.934 min), three possible compounds with a relative peak area of 12.29% were identified: n-hexadecanoic acid, L-(+)-ascorbic acid 2,6-dihexadecanoate, and pentadecanoic acid. Additionally, peak 37 at retention time 22.148 min showed three compounds with a considerable relative peak area of 11.80%: oleic acid, trans-13-octadecenoic acid, and cis-vaccenic acid (Table 3).

GC-MS analysis of the methanolic extracts of red and white cowpea revealed a diverse range of compounds belonging to multiple chemical classes, including alcohol derivatives, ammonia derivatives, esters, hydrocarbons, amino acids, alkaloids, free fatty acids, vitamins, carbohydrates, phenolics, lipids, terpenoids, and steroids. Among the fatty acids and their derivatives, several compounds exhibited notable biological activities. Dodecanoic acid, 3-hydroxy, methyl ester was reported to possess antifungal and antibacterial properties [43], while 10-octadecenoic acid, 9-octadecenoic acid (Z)-, and 2-hydroxy-1-(hydroxymethyl) ethyl ester demonstrated antimicrobial potential [44].

Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester exhibited antioxidant, anti-inflammatory, and antifungal activities [45], and n-hexadecanoic acid was associated with antioxidant and hypocholesterolemic

effects [46]. Hexadecanoic acid, methyl ester (methyl palmitate) displayed antibacterial activity [47], whereas trans-13-octadecenoic acid, methyl ester showed anti-inflammatory, dermatitogenic, and anaemiagenic properties. Oleic acid exhibited antifungal, anti-inflammatory, antioxidant, and antibacterial effects [48], while pentadecanoic acid, a saturated odd-chain fatty acid, has been linked to cardiometabolic, immune, and liver health benefits [49].

Nitrogen-containing compounds were also identified, including imidazole, 2-amino-5-[(2-carboxy)vinyl]-, which possesses antioxidant, antibacterial, and antifungal properties; aminoacetamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butyryl]- and heptadecanoic acid, 16-methyl-, methyl ester, both of which have been associated with rheumatoid arthritis treatment [50], and desulphosinigrin, which has demonstrated anticancer and antibacterial activities [51][52]. In addition, L-(+)-ascorbic acid 2,6-dihexadecanoate, classified as a provitamin C derivative, functions as an antioxidant [53]. Other bioactive compounds included L-gala-L-ido-octose, with antifungal and antibacterial activity [43], and cucurbitacin B, 25-desacetoxy, which is effective as both an anticancer and antibacterial agent [54].

Overall, the GC-MS profiling of red and white cowpea revealed a diverse spectrum of bioactive compounds, including fatty acids, nitrogen-containing molecules, vitamins, and other secondary metabolites with documented antioxidant, antimicrobial, anti-inflammatory, anticancer, and cardioprotective properties. The presence of these compounds not only supports the nutritional value of cowpeas but also highlights their potential as functional food ingredients and as promising candidates for pharmacological exploration. These findings

provide a strong scientific basis for further studies on the bioactivity, safety, and application of cowpea-derived compounds, thereby reinforcing the role of this underutilized legume in enhancing food security, public health, and local agricultural economies

Conclusion

This study demonstrates that indigenous cowpea varieties from Bulukumba, South Sulawesi possess significant nutritional and functional food potential. Both red and white cowpea varieties exhibited favorable nutritional profiles characterized by high carbohydrate and protein content with low lipid levels, indicating their suitability as alternative protein sources for human nutrition. The antioxidant activity, measured via DPPH assay, was markedly higher in red cowpea (2,115.33 $\mu\text{g/g}$) than in white cowpea (507.18 $\mu\text{g/g}$). GC-MS analysis revealed a greater diversity of bioactive compounds in the methanolic extract of red cowpea compared to that of white cowpea. These compounds belonged to various chemical classes, including alcohols, amines, esters, hydrocarbons, amino acids, alkaloids, free fatty acids, vitamins, carbohydrates, phenolics, lipids, terpenoids, and steroids, many of which have documented biological activities relevant to human health. The findings suggest that Bulukumba cowpea, particularly the red cultivar represents a valuable functional food resource with potential health promoting applications. Further research investigating the bioavailability and specific health benefits of these identified compounds would enhance the utilization potential of this indigenous legume crop.

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Author Contributions

Conceptualization, W.A and M.Z.A; methodology, W.A; formal analysis, W.A; investigation, W.A; resources, M.Z.A; data curation, W.A; writing—original draft preparation, M.Z.A; writing, review and editing, M.Z.A; visualization, W.A; supervision, M.Z.A; project administration, M.Z.A. Both authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethical Standards

This article does not contain any studies involving human or animal subjects.

Supplementary Information

The supplementary materials include:

Table S1. GC-MS analysis result showing identified compounds in red cowpea

Table S2. GC-MS analysis results showing identified compounds in white cowpea

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