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ANALYSIS OF NUTRITIONAL QUALITY AND BIOACTIVE COMPONENTS OF *CISSUS ROTUNDIFOLIA, CYPHOSTEMMA DIGITATUM* AND *CISSUS QUADRANGULARIS* (VITACEAE)

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ABSTRACT

Cissus rotundifolia, Cyphostemma digitatum, and *Cissus quadrangularis* which belong to Vitaceae family are used as local traditional vegetables by local communities in Saudi Arabia and different regions in Asia for nutritional and therapeutic purposes. They were analyzed in this study in order to provide scientific evidence for their usage as nutritional and medicinal herbs. The protein, fat, fibre, cyanogenic glycoside, phytic acid, flavonoid, and tannin contents of the three species differed significantly ($P < 0.05$). Results from the nutritional analysis revealed the richness of *C. rotundifolia* leaves in crude protein (14.54%), carbohydrates (52.32%), fats (6.99%), and energy value (330.35 Kcal/100 g). *C. rotundifolia* and *C. digitatum* leaves showed comparable amounts of both vitamins C and A. The highest crude fiber was found in *C. quadrangularis* (23.82%), which also exhibited a good carbohydrate content (57.20%). Calcium was the most abundant mineral in the studied whole leaf extracts, followed by potassium. *C. quadrangularis* contained the highest concentrations of sodium (236 mg/100 g), iron (29 mg/100 g) and the lowest level of magnesium (96 mg/100 g). The concentrations of heavy metals in all samples were below the permissible level in food. In terms of anti-nutritional factors, all extracts had comparatively low levels of cyanogenic glycoside, phytate, alkaloid, and saponin contents. The leaves of *C. quadrangularis* contained significantly high levels of phenol, flavonoid, tannin, and proanthocyanidin content. *C. rotundifolia* leaves had a high level of DPPH radical quenching power (IC_{50} = 52.27 µg/mL) that was comparable to that of the standard (IC_{50} = 51.47 µg/mL). Inhibitory activities against alpha-amylase significantly varied among the extracts analyzed. The leaves of *C. quadrangularis* strongly inhibited alpha-amylase, and their impact was close to the effect of acarbose as a standard anti-diabetic agent. The findings revealed that the studied leafy wild plants can be used as a good source of various nutrients and beneficial biochemicals.

Keywords: *Cissus rotundifolia, Cyphostemma digitatum*, *Cissus quadrangularis*, nutrients, anti-nutrients, bioactive compounds

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INTRODUCTION

Plants play a vital role in almost every culture on earth, and around 80% of people worldwide largely rely on herbs for their healthiness and healing (Esiyok *et al.*, 2004). Many native, wild edible plants are among-based sources that currently receive little attention from food scientists and nutritionists. The majority of wild food plants have low energy and good nutritional values (Trichopoulou *et al.*, 2000) high fiber (Reyes-García *et al.*, 2006), high antioxidant (Asano *et al.*, 2000), and low fat (Trichopoulou *et al.*, 2000) contents in compared to similar cultivated species. They have been ignored because people are unaware of their crucial health-promoting benefits (Bello *et al.*, 2019). Unfortunately, no well-defined studies are currently being conducted to support the consumption of historically known wild edible plants as sources of nutrition and therapeutics (Anwar *et. al.*, 2022). Many of them have been shown to have significant beneficial effects in fighting a variety of modern-day chronic diseases (Trichopoulou *et al.*, 2000; Finkel and Holbrook, 2000; Maritim and Sanders, 2003). Various plant species, which are used as wild green have therapeutic potential, so there is an overlap between these two kinds of plants (Heywood, 2011). It is important to investigate the nutritional value, phytochemistry, and potential health benefits of these plants.

Saudi Arabia has a rich history of folk medicine and is one of the most botanically diverse countries in the world. The majority of Saudis use herbal remedies either alone or in combination with traditional medicine (Almoshari, 2022). The use of wild plants as food is an integral part of the culture and tradition of certain indigenous communities in the country (Aati *et al.*, 2019).

The genus Cissus consists of 350 species, and at least a dozen plants from this genus have been traditionally used to treat various diseases. The locally available species of this genus in East and West Asia are commonly used to treat several health problems (Gabriel and Jameela, 2012). A variety of phytoconstituents have been identified in the genus Cissus which are biologically active as anti-allergic, anti-dyslipidemic, and hypoglycemic (Chan *et al.*, 2018).

C. rotundifolia is a nutritive wild plant used in different places and diverse cultures to nourish people in times of hunger and starvation. It is mainly rich in proteins, essential amino acids, sugars, dietary fibers, vitamins, and minerals (Al-Bukhaiti *et al.*, 2019). The plant is historically consumed as a leafy vegetable in Saudi Arabia. The fresh leaves are cooked to make a range of recipes with great nutritional content (Korish, 2016). Moreover, the plant is frequently utilized in herbal remedies, particularly for healing the liver, gastrointestinal ailments, and skin disorders (Alqahtani *et al.*, 2020).

C. digitatum is popular in Yemen, south of Saudi Arabia, and is commercially sold because it is frequently used to prepare various traditional meals (Al-Duais *et al.*, 2009 a) and as an appetite stimulant (Alasbahi and Groot, 2021). The processed and unprocessed plant samples have appropriate levels of phenols, carotenoids, provitamin A, and vitamins C and E (Al-Duais *et al.*, 2009 b). Furthermore, it is used as an herbal medicine in central Yemen to treat health problems such as headaches and, vomiting caused by malaria (Al-Duais *et al.*, 2009 a). The plant is rich in various phytoconstituents including phenols, flavonoids, tannins, alkaloids, saponins, and terpenoids (Khan *et al.*, 2016).

C. quadrangularis has nutritional benefits and has been used to treat a variety of health problems including, scurvy, gout, venereal disease, anorexia, syphilis, and menstrual disorders (Kelbore *et al.*, 2022), bone healing (Kaur *et al.*, 2022), hyperlipidemia, and overweight in diabetic rats (Syed *et al.*, 2022). In Saudi Arabia, fresh leaves of *C. quadrangularis* are extracted with margarine or olive oil and applied topically for the treatment of snake bites. For circumcision, fresh, softened leaves are applied directly to the wound (Abdel-Kader *et al.*, 2018). The plant has many biologically active components, including phenols, flavonoids, flavones, phytosterol, and alkaloids (Kaur *et al.*, 2022).

C. rotundifolia, *C. digitatum*, and *C. quadrangularis* are perennial, succulent climber wild plants belonging to Vitaceae family, widely distributed in southwestern Saudi Arabia. It is important to understand the phytoconstituents of these plants because they are still used in Saudi Arabian traditional medicine and food culture. Very little information has been documented about the nutritional values and bioactive components of these plants. Therefore, this research aimed to investigate the nutritional values, anti-nutritional factors, antioxidant properties, and anti-diabetic activity of these wild species in order to give scientific support for their use as dietary and therapeutic plants.

MATERIALS AND METHODS

Plant samples and extraction: The studied plants samples were collected from many locations in Al Baha region, southwestern Saudi Arabia, in November 2021. The three plants grow spontaneously and in abundance in valleys and on the edge of mountains and around cultivated lands climbing on different supports. Voucher specimens (BUH 98, BUH 90, BUH91) have been kept at the Herbarium of the Biology Department, Faculty of Science, University of Al-Baha, Al- Baha, Saudi Arabia. Fresh young leaves (about 30 days old) were air-dried at room temperature for 21 days before being ground into a coarse powder with an electric blender. A hundred g of each plant was soaked in 1000 mL of methyl alcohol (MeOH) at room temperature with agitation (130 rpm) using an orbital shaker for two days. The mixture was filtered using Whatman No.4 then the filtrate was evaporated to dryness using a rotary evaporator (Buchi, USA). The dry extract was taken for analyses of total polyphenols, total flavonoids, antioxidant capacity, and enzyme inhibitory activity.

Proximate analysis: Plant specimens were assayed for moisture, ash, crude protein, crude fat, and carbohydrate contents based on the procedures of the Association of Official Analytical Chemists (AOAC, 1995). For moisture content, samples were dried in an oven (Binder GmbH, Tuttlingen, Germany) at 105 ± 5°C until a constant weight was achieved. Ash content was determined by combusting dry leaves in a muffle furnace (Thermo Scientific, USA) at 600 ± 15 °C for 8 h. Crude protein (N = 6.25) was evaluated using the macro-Kjeldahl method. Crude fat was extracted in petroleum ether for 6 h using a Soxhlet apparatus and gravimetrically determined. Total carbohydrates were estimated by difference using proximate analysis measures. Soluble and insoluble dietary fibers were assessed by AOAC method (AOAC, 2005). Caloric value was estimated by the method described by Ihekoronye and Ngoddy (1985). Tests were done in triplicate, and the values were presented as g per 100 g.

Vitamin C content: The procedure outlined by Moreira *et al.* (2003) was used to evaluate the amount of vitamin C (L-ascorbic acid) in each sample. A plant sample (2 g) was mixed with 40 mL of oxalic acid (0.2%) solution. After filtration, 5 mL was taken and titrated with 2,6-dichloroindophenol. The data were given in mg per 100 g.

Carotenoids (provitamin A) determination: Carotenoid content (provitamin A) was determined in each sample by the assay outlined by Nemzer *et al.* (2020). Each plant specimen (0.4 g) was mixed with 10 mL of water-saturated butanol. After 15 min of shaking, the sample was covered with aluminum foil and left at room temperature for 60 min. The slurry was agitated again and left at room temperature for another 60 min, then centrifuged for 10 min at 4000 rpm. The absorbance rate was detected at 450 nm using a UV-Vis spectrophotometer (JENWAY, 6305, Japan). Total carotenoid content (mg/g) was calculated using lutein as a standard compound.

Mineral content

Microwave digestion: For mineral analysis, four mL of nitric acid (65%), 1 mL of hydrochloric acid (36%), 2 mL of hydrogen peroxide (30%), and 1 mL of deionized water were added to each plant specimen (0.25 g). The microwave's (Anton Paar Multiwave 3000 Microwave, Graz, Austria) digestion process was as follows: the power was first ramped up to 1400 watts for 10 min and held there for 20 min before being reduced to zero watts for zero min and held there for 15 min.

ICP-OES determination: Target elements were determined using an inductively coupled plasma optical emission spectrometer (ICP OES, Perkin Elmer, USA) equipped with a Meinhard Nebulizer type A. The instrumental conditions were as follows: 1300 W RF power, 15 L min^{-1} plasma flow, 0.2 L min^{-1} auxiliary flow, 0.8 L min^{-1} nebulizer flow, and 0.0015 L min^{-1} sample uptake rate. Argon (99%) was used as a carrier gas, and axial and radial views were used for element detection. A 2-point background was employed for determining the analytical signal. The standard solution (g L-1) was diluted with nitric acid (0.5%) to construct the calibration curves.

Anti-nutritional factors

Cyanogenic glycoside: The procedure outlined by Olopade and Onwuka (2005) was used for determining the cyanogenic glycoside content in the studied plants. A hundred mL of distilled water was added to 2.5 g of each sample, allowed to stand for 12 h at room temperature, and then filtered (Inuwa *et al.*, 2011). One g of picrate and 5 g of Na_2CO_3 were dissolved in 200 mL of distilled to prepare an alkaline picrate solution. Four mL of picrate solution were added to 1 mL of the filtrate, which was then left to stand in a water bath for 15 min. Rate of absorbance was read at 490 nm against a blank (1 mL of distilled water mixed with 4 mL of picrate solution). Different concentrations of KCN (0.1 – 1.0 mg/mL) were used for constructing the standard curve. The cyanogenic glycoside content in the tested specimens was determined from the constructed standard curve.

Saponin content: The modified method by Hudson and El-Difrawi (1979) was adopted for measuring the saponin content in the studied plants. Ten g of each plant were mixed well in 200 mL of 20% aqueous ethanol. The slurry was stirred for 12 h at 55 ºC using a magnetic stirrer, then filtered. The residues were re-extracted using aqueous ethanol (200 mL, 20%). The combined filtrates were concentrated to 40 mL under vacuum, then placed in a 125-mL separating funnel. Twenty mL of diethyl ether were mixed well with each extract. The ether layer was removed, and the aqueous solution's pH was adjusted to 4.5. After shaking with n-butanol (60 mL), the solution was washed with an aqueous solution of sodium chloride (10 mL, 5%), dried, and then weighed.

Phytic acid content: The amount of phytate in each sample was evaluated according to the procedure outlined by Latta and Eskin (1980). Ten mL of 2.4% HCL were added to 0.1 g of plant sample and then centrifuged at 3000 rpm for 30 min. One mL of Wade reagent (0.03% Fe $CL_3.6H_2O$ and 0.3% sulfosalicylic acid in water) was mixed well with 3 mL of each plant material. The absorbance of the clear supernatant was read at 500 nm using a UV-Vis spectrophotometer. The concentration of phytic acid in each suspension was determined from the constructed calibration curve of sodium phytate.

Determination of alkaloid: The percentage of alkaloid was assessed in plant samples by Essack *et al.* (2017) method. Five g of plant samples were mixed with acetic acid (200 mL, 10%), and the slurry was left to stand at room temperature for 4 h. The mixture was filtered and concentrated to 50 mL using a rotary evaporator at 60 ºC. A concentrated solution of NH_4OH (1 mL) was added dropwise to the slurry until the precipitate was completely formed. After settling, the precipitated was collected, washed (water and ammonium hydroxide), filtered, dried at room temperature then weighed.

Bioactive constituents

Determination of total polyphenols: Total polyphenol was quantified using Folin-Ciocalteu"s phenol reagent (Velioglu *et al.*, 1998). To 1.5 mL of Folin-Ciocalteu (1: 10 with deionized water), one mL of each extract (10 mg/mL) was added. The slurry was allowed to stand for 5 min at 30 ºC before 1.5 mL of Na_2CO_3 (6%) was added. After 90 min, the absorbance was measured at 725 nm using a UV-vis spectrophotometer. For calculating the concentration of total polyphenol, gallic acid was used as a standard to construct the calibration curve. Values were given as mg gallic acid equivalent (GAE)/g dry weight (DW).

Determination of total flavonoids: The spectrophotometric procedure outlined by Chang *et al.* (2002) was adopted for determining the concentration of flavonoids in each specimen. One mL of each specimen

(10 mg/mL) was mixed well with methanol (3 mL), aluminum chloride (0.2 mL, 10%), potassium acetate (0.2 mL, 1 M), and distilled water (5.6 mL). After 30 min at 30 ºC, the rate of absorbance was detected at 415 nm using a UV-vis spectrophotometer. A calibration curve was constructed, and the data were presented as mg Quercetin equivalent (QE)/g DW.

Determination of condensed tannin content: Adamu *et al.* (2022) method was used for estimating the tannin content in the studied plants. To 1 g of plant sample, 10 mL of 1% HCL in methyl alcohol were added, and the mixture was shaken at 80 rpm for 24 h at room temperature. The extract was centrifuged at 100 rpm for 5 min, then 5 mL of Vanillin-HCL reagent were added to 1 mL of the supernatant. The rate of absorbance was detected at 500 nm using a UV-Vis spectrophotometer. The calibrated curve of tannic acid (TA) was used to calculate the tannin content (mg TA/ g DW) in each suspension.

Determination of total proanthocyanidin content: The assay outlined by Mangoale and Afolayan (2020) was used for determining the total proanthocyanidin content in each sample with some modifications. One mL sample (10 mg/mL), 6 mL of vanillin-methanol solution (4%), and HCL (3 mL) were mixed using a vortex. The slurry was left at 30 ºC for 15 min. The absorbance was detected at 500 nm using a UV-vis spectrophotometer. The level of proanthocyanidin was determined from the calibrated curve of catechin (CA) and presented as mg CE/g DW.

Antioxidant assays: The antioxidant capacity of each sample was assessed by DPPH (2,2 Diphenyl-1 picrylhydrazyl), $ABTS^{+\bullet}$ [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] and FRAP (ferric reducing antioxidant power). Vitamin C (ascorbic acid) was taken as a standard antioxidant.

DPPH assay: Plant extracts' radical scavenging ability was measured using the DPPH method (Braca *et al.*, 2001). Three mL of 0.004% DPPH in methanol was added to a plant sample (0.1 mL, 3.125–100 g/mL). After shaking, the suspension was left at 30 ºC for 30 min. Methanol plus DPPH solution was used as a negative control (Ac). The absorbance rate of each test was read at 517 nm against MeOH as a blank (As). The percentage of inhibition of DPPH was detected from the equation below:

inhibition (%) = [Ac – As)/Ac] × 100.

The IC_{50} was calculated from the constructed calibration curve.

$ABTS^{+\bullet}$ assay: The $ABTS^{+\bullet}$ assay (Moreno-Montoro *et al.*, 2015) was adopted for determining the antioxidant capacity of each sample. The $ABTS^{+\bullet}$ radicals were generated by combining ABTS diammonium salt (0.35 mL, 7.4 mmol/L) with potassium persulfate (0.35 mL, 2.6 mmol/L). The slurry was allowed to stand in a dark room at 30 ºC for 15 h. The prepared ABTS solution was diluted with 95% EtOH (1:40, v/v) and taken as a control (Ac). A plant sample (0.2 mL, 10 mg/mL) was mixed well with $ABTS^{+\bullet}$ solution (2 mL) and left at 30 ºC for 20 min. The absorbance was detected at 734 nm (As) and the ability of the plant sample to reduce the $ABTS^{+\bullet}$ radicals was determined as follows: inhibition (%) = [1 – As/Ac] × 100.

The IC_{50} was determined from the calibrated curve.

FRAP assay: The described assay by Idris *et al.* (2017) was adopted to assess the ferric-reducing antioxidant power of each crude sample. A one mL sample (different concentrations) was added to a mixture of 0.2 M phosphate buffer (2.5 mL, pH 6.6) and 1% $K_3[Fe(CN)_6]$ (2.5 mL) and then left at 50 ºC for 20 min. After the addition of 10% trichloroacetic acid (2.5 mL), the reaction mixture was centrifuged at 3000 rpm for 10 min. The obtained supernatant (2.5 mL) was added to a mixture of distilled water (2.5 mL) and 0.1%ferric chloride (0.5mmL). Increase in the absorbance was read at 700 nm using a UV-vis spectrophotometer.

Alpha-amylase inhibitory assay: The inhibitory effect of the crude samples on the alpha-amylase enzyme was tested according to the assay adopted by Sangeetha and Vedasree (2012). Plant samples and acarbose as a reference standard (100 µL) at different concentrations were mixed with 200 µL of the alpha-amylase enzyme (1 unit mL^{-1}) and 100 µL of phosphate buffer (2 mM, pH 6.9) and incubated for 20 min. After the addition of starch solution (100 µL, 1%), the slurry was left at 30 ºC for 5 min. The same was performed for the control where 200 µL of the enzyme was replaced by the buffer. 0.5 mL of DNS reagent (1% 3,5-dinitrosalicylic, 12% $C_4H_4Na_2O_6$ in 0.4 mol L^{-1} NaOH) was added to both sample and control then immersed in a boiling water bath for 15 min. After cooling, the α-amylase activity was detected at 540 and the percentage inhibition of α-amylase was determined from the formula: Inhibition (%) = 100 [control – test/ control]. IC_{50} values were determined for each sample by linear regression analysis.

Statistical analysis: For statistical analysis, one-way analysis of variance (ANOVA) and Tukey multiple comparison tests were performed (the significant level was $P < 0.05$) using GraphPad Prism 5.0. The obtained values were shown as means with standard deviations.

RESULTS AND DISCUSSION

Proximate results: The nutritional components of the investigated wild plants are summarized in Table 1. *C. rotundifolia* had a high moisture content, but there was no

significant difference ($P < 0.05$) in moisture content between *C. digitatum and C. quadrangularis.* Both Korish (2016) and Al-Bukhaiti *et al.* (2019) revealed high moisture levels in *C. rotundifolia* leaves (93.1 ± 0.2 and 81.49 ± 0.29%, respectively). Compared to the values of this study, Anju *et al.* (2022) demonstrated that the moisture content in leaves of *C. quadrangularis* was extremely low (56.64%). According to the study performed by Lakshmanan *et al.* (2021), the stem of *C. quadrangularis* contains 84% of water. Moisture content aids in the preservation of protoplasmic content in cells and leaf texture, both of which are essential for plants' metabolic function (Ooi *et al.*, 2012). Consumers are very concerned about water since food's moisture level is linked to chemical degradation and microbial contamination (Arasaretnam *et al.*, 2018).

Table 1: Proximate composition and energy value of *C. rotundifolia C. digitatum* and *C. quadrangularis* leaves (dry weight basis).

Analyte	*C. rotundifolia*	*C. digitatum*	*C. quadrangularis*
Moisture (%)	86.39 ± 0.64[a]	84.57± 0.4180[b]	78.29 ± 0.6787[c]
Ash (%)	12.92 ± 0.23[a]	9.58 ± 0.75[b]	9.57 ± 0.59[b]
Crude protein (%)	14.54 ± 0.43[a]	11.11 ± 0.59[b]	6.58 ± 0.40[c]
Crude fat (%)	6.99 ± 0.41[a]	4.89 ± 0.24[b]	2.83 ± 0.23[c]
Crude fibre (%)	13.41 ± 0.53[a]	17.65 ± 0.64[b]	23.82 ± 0.76[c]
Carbohydrate (%)	52.32 ± 0.40	56.77 ± 0.56	57.20 ± 0.50
Energy (Kcal/100 g)	330.35	315.53	280.59
Vitamin C (mg/100 g)	0.54 ± 0.05[a]	0.53 ± 0.06[a]	0.07 ± 0.01[b]
Vitamin A Provitamin A (mg/100 g)	0.62 ± 0.06[a]	0.62 ± 0.04[a]	0.015 ± 0.00[b]

Values are given as average ± standard deviation. Means with different letters within a same row differ significantly (Tukey's Multiple Comparison Test, $P < 0.05$).

Ash content was high in *C. rotundifolia* leaves, and with high ash content, one could expect that *C. rotundifolia* leaves could contain a substantial amount of minerals. However, the amount of ash in *C. rotundifolia* leaves was nearly identical to that (12.53%) reported by Al-Bukhaiti *et al.* (2019). On the other hand, it was lower than that (16.30 ± 0.2%), as stated by Korish (2016). No significant difference was noticed in the ash content between *C. digitatum and C. quadrangularis* leaves. When compared to the level (17.08%) recorded by Anju *et al.* (2022), the percentage of ash in the leaves of *C. quadrangularis* in the present work was very low.

The three plant samples had significantly ($P < 0.05$) different crude protein concentrations. The amount of crude protein in the leaves of *C. rotundifolia* was high compared with that in the leaves of the other two plants, and the result (12.16%) recorded by Al-Bukhaiti *et al.* (2019), and the value (12.5 ± 0.1%) obtained by Korish (2016). This suggests that *C. rotundifolia* leaves may serve as a promising source of affordable plant proteins. According to Kaur *et al.* (2022), proteins were qualitatively detected in ethanol and chloroform extracts of *C. quadrangularis*. The presence of 9.88 mg per g fresh weight of *C. quadrangularis* leaves has been reported by Anju *et al.* (2022). In comparison to the range of protein percentages (13.25-26.44%) recorded by Afolayan and Jimoh (2009) for *Chenopodium album*, *Sonchus asper*, *Solanum nigrum* and *Urtica urens* as leafy vegetables, the range of protein content in the analyzed plants' leaves was noticeably low. However, for vegetarians, both *C. rotundifolia* and *C. digitatum* leaves, which have high crude protein contents, can be used as a cheap source of protein. In addition, they may, in general, and to a certain extent, help fill the protein gap in human diets and be beneficial in preventing protein-energy malnutrition.

The three plants' fat contents showed significant differences. *C. rotundifolia* dominated the three plants in terms of fat content followed by *C. digitatum* and *C. quadrangularis*. The detected fat in *C. rotundifolia* leaves was comparable to that reported (7.45%) by Korish (2016). It was found that the crude fat content of *C. rotundifolia* leaves was more than three times greater than that reported for the same plant (2.77%) by Al-Bukhaiti *et al.* (2019). As a result, *C. rotundifolia* leaves could be a good source of oil and fatty acids, which are important for maintaining good health. Consuming these plants could therefore help the body gain healthy fat. However, fat is one of crucial ingredients in food (Satter *et al.*, 2016), which offers around 9 calories from one gram. *C. quadrangularis* leaves showed a low amount of crude fat as compared with the other two plants. In contrast to the finding of this investigation, the crude fat content of *C. quadrangularis* leaves (5.79%) evaluated by Anju *et al.* (2022) was high.

The results obtained for crude fiber analysis indicated that *C. quadrangularis* leaves had an extremely high amount of fiber. However, high dietary fiber can help with digestion, lower blood cholesterol, and lower the cancer risk in the colon (Al-Farga *et al.*, 2016). The lowest quantity of crude fiber was found in the leaves of *C. rotundifolia*, which was relatively lower than the amount (14.10%) reported by Al-Bukhaiti *et al.* (2019), and noticeably greater than the amount (8.43%)

demonstrated by Korish (2016). The range of fiber content in the present study was high compared with that (0.39-1.79%) reported in fourteen different wild leafy vegetables evaluated by Bvenura and Afolayan (2016). This variation in fiber content could be due to plant type, plant age and environmental and genetic factors.

Slight variations were observed among the three plant samples in their carbohydrate contents. The carbohydrate level in the leaves of *C. rotundifolia* was found to be lower than the finding (72.54%) reported by Al-Bukhaiti *et al.* (2019). A phytochemical analysis (Khan *et al.*, 2016) showed the presence of carbohydrates and reducing sugars in the root and stem of *C. digitatum*. According to Dhanasekaran's study (2020), no carbohydrates were found in the ethanol and methyl alcohol extracts of the aerial parts of *C. quadrangularis*. On the other hand, the detected level of carbohydrates in the fresh leaves of *C. quadrangularis* by Anju *et al.* (2022) was 20.7 mg/g.

The calorific values varied among the studied dry leaf samples. This variation in energy values among the investigated plants might be due to the significant variation in fat contents, or it could be attributed to environmental factors, among other factors. The high energy values of the investigated plants suggest that they can be utilized in the formulation of various dietary supplements.

The tested three plants had reasonable levels of vitamin C and pro-vitamin A, both of which are important micronutrients in diets. A higher level of vitamin C was found in the leaves of *C. rotundifolia* and *C. digitatum*, and a very low amount was detected in *C. quadrangularis*. The amounts of vitamin C reported by Al-Bukhaiti *et al.* (2019) and Al-Duais *et al.* (2009 b) in the leaves of *C. digitatum* were 0.02 mg/100 g and 49.5 mg/100 g, respectively, which were lower than the result of this study. The vitamin C contents of the three plants were incomparable to the values reported by Datta *et al.* (2019) for pumpkin (3.47-4.39 mg/100 g), tomato (23 mg/100 g), and spinach (51 mg/100 g). When compared to vitamin C content (2.14-156.92 mg/100 g) of twenty leafy vegetables examined by Pan and Bhatt (2018), the levels of vitamin C identified in the investigated leaf samples were relatively low. High levels of pro-vitamin A in *C. rotundifolia* and *C. digitatum* indicate the potential of using these plants' leaves as a reliable source of this vitamin, especially in the developed areas of the world. The amount of provitamin A in *C. quadrangularis* was low and significantly different from the amounts in the other two plants. The richness of *C. digitatum* in provitamin A among other food ingredients and micronutrients was reported by Al-Duais *et al.* (2009 b).

Mineral content: The concentrations of elements in the plant samples are depicted in Table 2. Clear variations were observed among the examined plants in their metal contents and contained a wide range of concentrations of both macro minerals and beneficial trace elements. Variations in chemical composition among the studied plants may be due to age of the plant at harvest time, soil composition, rate of uptake of minerals by individual plants among other factors (Anjorin *et al.*, 2010). Calcium was the predominant element present in the plants studied. The investigated samples contained substantial quantities of Ca, Mg and Fe in their leaves. Thus, they might have a significant impact on addressing the nutritional needs of these elements in food, especially in rural populations. *C. rotundifolia* and *C. digitatum* leaves contained nearly equal amounts of both calcium and magnesium. The two plants can supply the recommended daily allowance (RDA) for calcium and magnesium when the RDA for minerals is considered (Afolayan and Jimoh, 2009; Steyn *et al.*, 2001). High quantities of iron were detected in leaves of the investigated plants, indicating that they might be a good source of iron since the daily requirement is 18 mg/100 g (Afolayan and Jimoh, 2009) and 10 mg/100 g (Steyn *et al.*, 2001). The concentration of Na was high in *C. quadrangularis* leaf samples while a remarkably high quantity of K was detected in *C. rotundifolia* leaves.

Table 2: Mineral contents of *C. rotundifolia C. digitatum* and *C. quadrangularis* leaves (mg/100 g dry weight basis)

	C. rotundifolia	*C. digitatum*	*C. quadrangularis*	RDA[a] (mg/day)	RDA[b] (mg/day)	Permissible level in food[c] (mg/100 g)
Ca	1050	1066	689	1000	800	
Mg	572	582	96	400	120	
Fe	19	25	29	18	10	
Na	112	89	236		300	
K	985	791	543		1400	
Pb	0.01	0.009	0.09			0.03
Cd	0.006	0.008	0.06			0.02
Cu	5.7	4.2	5.8			7.33
Zn	1.2	3.4	2.3			9.94
Ni	2.7	3.1	4.6			6.69
Co	1.9	1.8	2.1			5

[a]Afolayan and Jimoh (2009), [b]Steyn *et al.* (2001), [c]Codex Alimentarius commission (2001).

Variation in trace and heavy element (Pb, Cd, Cu, Zn, and Co) contents was observed among the plant samples. This variation could be related to atmospheric pollution, vehicular emissions, level of trace elements in the soil and anthropogenic activities among other factors (Kananke *et al.*, 2016). Leafy vegetables are well known to accumulate heavy metals. As a result, contamination may happen if plants are grown in contaminated soil (Kananke *et al.*, 2016). To ensure consumer safety, the calculated heavy metal concentrations in the studied samples were compared to permissible limits proposed by Codex Alimentarius commission (2001). The concentrations of Pb and Cd in the leaves of *C. quadrangularis* were higher than the values that are safe for human consumption, while the amounts of these metals in the other two plant samples remained below the permitted limits. The detected levels of Cu, Zn, and Co in all plant samples were within the allowable maximum concentrations of these elements in the food. Lead and zinc in all plant samples were below the maximum permissible level reported by Egwu *et al.* (2022) in vegetables. These findings have increased the possibility that wild vegetable plants could be used in concentrated form as a good source of essential and trace elements in dietary supplements.

The element contents of the studied plants were incomparable to the values demonstrated by Korish (2016) and Al-Bukhaiti *et al.* (2019) for *C. rotundifolia* leaves. The presence of essential macro elements (Na, K, Ca, Mg, and P) and microelements (Fe, Zn, and Cu) at different concentrations have been reported in the fruits of *C. rotundifolia* (Hegazy *et al.*, 2019). Lakshmanan *et al.* (2021) had found that *C. quadrangularis* stem contained sufficient levels of both K and Mg. When comparing the mineral quantities obtained in this study with the Recommended Dietary Allowances values, the results revealed that the tested plant samples may be a good supplement for some of the examined elements. Moreover, the study's findings suggest that the studied leaves are rich in certain essential elements needed for human intake and are thus suitable for introduction into cultivation.

Anti-nutritional factors: Table 3 presents the antinutritional factors detected in the plant samples. The level of these antinutrients varied across the examined plant leaves. The highest levels of the antinutrients were recorded in the leaves of *C. quadrangularis*. Statistically significant variations ($P< 0.05$) in cyanogenic glycoside contents were observed among the leaf samples. The level of cyanogenic glycoside in *C. rotundifolia* leaves closely agreed with the result (0.023 mg/100 g) obtained by Korish (2016). The levels of cyanogenic glycoside in the investigated plant leaves were within the permissible range of 5.3-80 mg/100 g suggested by Wobeto *et al.* (2007).

Table 3: Antinutritional factors in leaves of *C. rotundifolia*, *C. digitatum* and *C. quadrangularis*

Analyte	*C. rotundifolia*	*C. digitatum*	*C. quadrangularis*
Cyanogenic glycoside (mg/100 g)	0.029 ± 0.002^{c}	0.6167 ± 0.09^{b}	1.52 ± 0.09^{a}
Alkaloids (%)	0.013 ± 0.003^{b}	0.027 ± 0.002^{b}	0.073 ± 0.015^{a}
Phytic acid (mg/100 g)	89.29 ± 1.05^{c}	94.43 ± 1.13^{b}	104.5 ± 0.59^{a}
Saponin (%)	9.133 ± 0.60^{b}	9.11 ± 0.44^{b}	11.60 ± 0.92^{a}

Values are given as average ± standard deviation. Means with different letters within a same row differ significantly (Tukey's Multiple Comparison Test, $P < 0.05$).

C. rotundifolia and *C. digitatum* showed no significant differences in their alkaloid contents. Alkaloid concentration in *C. digitatum* and *C. quadrangularis* was above the threshold (0.02%) in vegetables (Egwu *et al*., 2022), while that in *C. rotundifolia* was below the threshold level. The presence of alkaloid in the stem and root of *C. digitatum* have been reported by Khan (2016). Alkaloids have a variety of physiological uses, and can be harmful to humans, and are widely used in medicine for drug synthesis (Egwu *et al.*, 2022). They have beneficial health effects, but on the other hand, they have been shown to interfere with nerve impulse transmission and can interact with cellular components, leading to many health problems (Koleva *et al.*, 2012).

The phytic acid concentrations in the tested plant samples were found to be substantially lower than the values provided by FAO (1990). The amount of phytic acid found in *C. rotundifolia* leaves was extremely higher than the amount (0.76 mg/100 g) reported by Korish (2016). The determined phytic acid in *C. quadrangularis* was markedly higher than the amount (20 mg/100 g) observed by Rex and Ravi (2020). However, for vegetarians, the average daily consumption of phytic acid is 2000-2600 mg, whereas for rural populations in developing countries who eat mixed diet, it is 150-1400 mg (Reddy, 2001). A high phytate content in foods is nutritionally significant not only because phytate phosphorus is unavailable to humans, but also because it reduces the availability of certain dietary elements like iron and zinc (Siddhuraju and Becker, 2001). As a result, the low values of phytic acid obtained in this study are expected to improve the bioavailability of protein and nutritional elements for the consumers of the studied plants.

A relatively moderate level of saponin was observed in all studied plant samples. Saponin levels were nearly equal in *C. rotundifolia* and *C. digitatum* leaves. The concentrations of saponins in all plant samples were found to be higher than the detected amounts in some South Africa wild leafy vegetables (Afolayan and Jimoh, 2009). However, the moderate level of saponins in vegetables should not cause a problem if properly processed, because processing reduces the amount of antinutrients to acceptable levels (Abifarin *et al.*, 2021). Saponin has potential health benefits and harmful effects. It can inhibit the metabolic pathways and absorption of minerals, protein, and starch (Thompson, 1993).

Based on previous studies, traditional leafy vegetables have antinutritional components which are known to substantially reduce their nutritional benefits. Over a long period, consuming large quantities of these components could have negative health effects (Sivakumar *et al.*, 2018). However, when leafy vegetables are treated in particular ways, such as blanching and cooking, some of these antinutrients are reduced (Ejoh *et al.*, 2017).

Bioactive compounds: Table 4 displays concentrations of the bioactive compounds (total polyphenols, total flavonoids, condensed tannins, and total proanthocyanidins) in the leaves of the studied plants. The three plant samples showed significant variations ($P < 0.05$) in their total flavonoid, and condensed tannin contents. No significant differences were seen between *C. rotundifolia* and *C. digitatum* in their total polyphenol and total proanthocyanidin contents. The total polyphenol and proanthocyanidin contents *of C. rotundifolia* and *C. digitatum* did not significantly differ. In comparison to the other bioactive compounds, the amount of tannins in the investigated plant samples was extremely low. *C. quadrangularis* leaves had a significantly ($P < 0.05$) greater amount of both total polyphenols and total flavonoids as compared to the leaves of the other two species. This finding suggests that *C. quadrangularis* leaves can be used as a promising healthy source of phenols and flavonoids.

Table 4: Bioactive components in leaves of *C. rotundifolia*, *C. digitatum* and *C. quadrangularis*

Analyte	*C. rotundifolia*	*C. digitatum*	*C. quadrangularis*
Total polyphenols (mg GAE/ g)	9.4 ± 0.75^{b}	10 ± 0.47^{b}	13 ± 0.55^{a}
Total flavonoids (mg QE/ g)	3.2 ± 0.40^{c}	6.3 ± 0.46^{b}	12 ± 0.95^{a}
Condensed tannins (mg TA/ g)	0.13 ± 0.05^{c}	0.31 ± 0.05^{b}	0.60 ± 0.08^{a}
Total proanthocyanidins (mg CA/ g)	4.4 ± 0.59^{b}	5.1 ± 0.31^{b}	6.7 ± 0.50^{a}

Values are given as average ± standard deviation. Means with different letters within a same row differ significantly (Tukey's Multiple Comparison Test, $P < 0.05$).

The amount of total polyphenols found in the leaves of *C. rotundifolia* was much lower than the amount (26.8 mg GAE/g DW) found by Alzoreky and Nakahara (2001), but higher than the values (3.18 and 7.61 mg GAE/g DW) demonstrated by Said *et al.* (2015) and Al-Bukhaiti *et al.* (2019), respectively. The level of total flavonoids and condensed tannins in *C. rotundifolia* was greater than the amounts of flavonoids (1.35 mg /g DW) and tannins (0.26 µg/100 g DW) reported by Said *et al.* (2015) and Korish (2016), respectively.

The presence of phenols and flavonoids in the methanol extracts of roots and stems of *C. digitatum* had been revealed by Khan *et al.* (2016). The concentration of total polyphenols in methanol extract of *C. digitatum* leaves in this study was less than the amount of total phenolics in the water (14.1 mg GAE/g) and ethyl acohol (19.5 mg GAE/g) extracts reported by Al-Duais *et al.* (2009a) for the same plant. This variation in total polyphenols could be due to the polarity of the solvents or biological, environmental, and technical factors (Ksouri *et al.*, 2008). According to Alasbahi and Groot (2021), the leaves of *C. digitatum* contained phenolic compounds as well as other components that could be beneficial to livestock. Tannin had been detected in both roots and stems of *C. digitatum*, among other secondary metabolites Khan *et al.* (2016). The content of total polyphenols and total flavonoids in *C. quadrangularis* was relatively higher than the levels of polyphenols (10.65 mg GAE/g DW) and flavonoids (10.79 mg QE/g DW) reported by Anju *et al.* (2022).

Antioxidant capacity: The calculated IC_{50} values of each plant extract and ascorbic acid as a reference antioxidant are summarized in Fig. 1. Different levels of antioxidant activity were found among the examined plant samples using the three confirmatory antioxidant assays. It was evident from the three analytical methods that the plant extracts' antioxidant capacities varied significantly ($P < 0.05$). In contrast to the other two extracts, the methanol extract of *C. rotundifolia* demonstrated the highest DPPH and FRAP antioxidant activity. In comparison to ascorbic acid as a reference compound, the extract of *C. rotundifolia* was found to have strong DPPH free radical scavenging action. The ability of the reference compound and the *C. rotundifolia* extract to scavenge DPPH free radicals did not differ significantly. The antioxidant activity of *C. rotundifolia* against the ABTS free radicals

and that of *C. quadrangularis* toward DPPH free radicals were nearly equivalent. The antiradical activity against ferric ion reducing antioxidant power of *C. rotundifolia* extract was comparatively good when compared with that of ascorbic acid as a standard antioxidant compound. Al-Bukhaiti *et al.* (2021) stated that the MeOH extract of *C. rotundifolia* had DPPH and ABTS radical scavenging activity of IC_{50} = 0.475 and 0.79 mg/mL, respectively. These findings did not agree with those of this study. The variation in results could be attributed to the effect of the extraction conditions or to the antioxidant contents of each species (Al-Bukhaiti *et al.*, (2019). Hegazy *et al.* (2019) have reported that *C. rotundifolia* contains important amounts of bioactive compounds that have high antioxidant properties. The trolox equivalent antioxidant capacity (TEAC) of a methanol extract from *C. rotundifolia* had been previously reported by Alzoreky and Nakahara (2001) using the ferrylmyoglobin /ABTS assay. Said *et al.* (2015) demonstrated that methanol extracts of *C. rotundifolia* at different concentrations (20, 50, 100, 150, and 200 mg/mL) had the capability to reduce the DPPH free radicals. *C. rotundifolia* had the highest antioxidant activity among the three plants in the DPPH assay, which indicates that it has health-promoting properties. The high phenol and flavonoid contents of *C. rotundifolia* leave*s* may be responsible for their high DPPH radical scavenging activity (Prasad *et al.*, 2005).

Khan *et al.* (2016) found that the methanol extract of *C. digitatum* roots had a high antioxidant activity (93.518%) against DPPH free radicals. Bello *et al.* (2019) reported that *C. adenocaule* root, stem bark, and leaf extracts had different antioxidant activities. Al-Duais *et al.* (2009b) attribute the high antioxidant activity of *C. digitatum* leaves to their remarkable concentrations of vitamin C, vitamin E, and carotenoids. Since ancient times, scientists have known that the antioxidant properties of vitamins C, E, and carotene help cells protect themselves against reactive oxygen species, which can harm DNA (Hunter and Willett, 1994). According to Al-Duais *et al.* (2009a), it is possible to easily obtain high antioxidants from *C. digitatum* fractions for use in food and other applications because of the high yield and the ease of *C. digitatum*'s growth, processing, and preservation. According to Dhanasekaran (2020), the reported IC_{50} values for both ethanolic (101.4 ± 6.3 µg/mL) and methanolic (114.1 ± 5.8 µg/mL) extracts of *C. quadrangularis* areal parts using the DPPH assay were higher than the finding of this study. In contrast, the antioxidant activities of the ethanol (IC_{50} = 64.5 ± 3.8 µg/mL) and methanol (IC_{50} = 67.1 ± 4.3 µg/mL) extracts using the FRAP method were less than the result of this investigation.

The DPPH radical scavenging activity result (IC_{50} = 1.097 mg/ml) obtained by Anju *et al.* (2022) for a methanol extract of *C. quadrangularis* was disagreed with the result of this study. Murthy *et al.* (2003) revealed that methanol and aqueous extracts of *C. quadrangularis* stem had less significant antioxidant activity than ethyl acetate extract, while n-hexane extract had the lowest level of antioxidant capacity. According to Rex and Ravi (2020), the methanol extract of *C. quadrangularis* stem exhibits significant antioxidant and free radical rummaging activity in both *in vivo* and *in vitro* systems, and it also inhibits lipid peroxide production in erythrocytes, superoxide radical production, and DPPH free radical formation. The tested plant samples were found to exhibit potent antioxidant activities, which could account for and support some of their traditional medicinal applications. However, the obtained antioxidant results of the methanol extract of the three plants indicate their health promoting benefits.

Alpha-amylase inhibitory results: Leaf extracts of the three species at a concentration of 10-160 µg/ mL were tested for antidiabetic activity using the alpha-amylase assay. Throughout each experiment, the test samples containing varied concentrations of plant extract were compared to control samples made in the same manner but without any plant extract. The positive standard inhibitor utilized was acarbose. The inhibitory effects of MeOH extracts of the leaves of *C. rotundifolia, C. digitatum* and *C. quadrangularis* as well as acarbose as a reference inhibitor on α-amylase are displayed in Fig. 2A. The findings demonstrated that the extracts' inhibitory activity was a concentration-dependent process, which indicated that as concentration increased, so did the percentage of inhibition. As a result, the reported inhibitory activities were investigated further, and their IC_{50} values were determined.

C. quadrangularis extract had a high inhibitory percentage when compared with acarbose as a standard inhibitor. The extract of *C. rotundifolia* showed moderate inhibitory activity, while that of *C. digitatum* had very low activity. The calculated half maximal inhibitory concentration (IC_{50}) values of alpha-amylase for *C. rotundifolia, C. digitatum, C. quadrangularis* extracts, and acarbose are illustrated in Fig. 2B. *C. quadrangularis* extract exhibited strong inhibitory activity against alpha-amylase, which was relatively comparable with the positive control. According to this finding, it might be able to extract potent anti-diabetic components from *C. quadrangularis* leaves. The extremely low inhibitory activity of *C. digitatum* leaves was demonstrated by their extraordinarily high IC_{50} value in comparison to the other extracts and the positive control. Onyechi and Ibeanu (2016) proposed that the flour from *C. rotundifolia* has biologically beneficial properties that can be used to treat non-insulin-dependent diabetes mellitus. According to Alkhateeb (2022), the natural food made from *C. digitatum* has anti-diabetic properties when used to treat a rat model of diabetes.

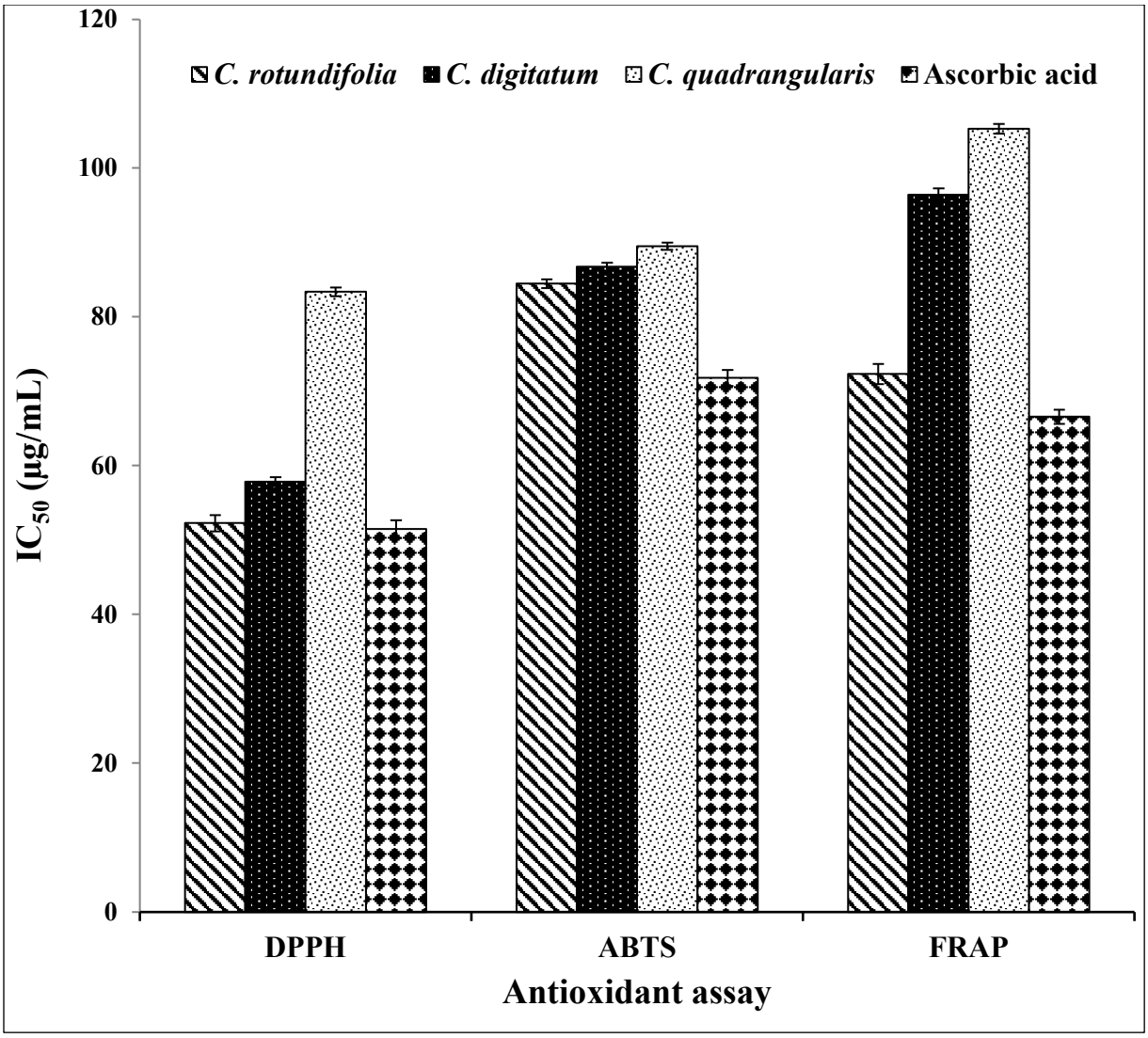

Fig. 1: Antioxidant activity of methanol extract from *C. rotundifolia, C. digitatum* and *C. quadrangularis* and ascorbic acid as a reference antioxidant. Results are means ± SD (n = 3).

The finding of the present investigation suggests that the extract of *C. quadrangularis* leaves, which exhibited the highest inhibitory activity, could possibly be used as a natural ingredient to reduce body mass and glucose levels in the blood. Most likely, the extract of *C. quadrangularis* leaves contained a high amount of substances that may compete with the substrate for binding to the enzyme's active site (Rahimzadeh *et al.*, 2014). So, it is important to conduct a thorough scientific analysis of *C. quadrangularis* leaf extract's efficacy as an alpha-amylase inhibitor. The results reported by Lee *et.al.* (2018) suggested that *C. quadrangularis* extract may have an anti-obesity effect by decreasing the expression levels of adipogenesis and lipogenesis-related genes and proteins. According to Sharp *et al.* (2007), aqueous extracts of *C. quadrangularis* stems and leaves have flavonoids and stilbenes, which decrease the activity of amylase, lipase, and glucosidase enzymes. Zaki *et al.* (2021) demonstrated that at concentrations of 250 and 300 µg/mL, the methanol extract of *C. quadrangularis* stems showed moderate inhibition of porcine pancreatic alpha-amylase.

Several studies have shown that many herbs possess the potential to be reliable sources of antidiabetic agents. Polysaccharides, terpenoids, steroids, alkaloids, glycopeptides, and polyphenols, such as chalcones, flavans, anthocyanins, hydroxycinnamic acid, and epicatechin, are among the active components in these plants (Khanal and Patil, 2020; Peddio *et al*., 2022). Different kinds of studies have been conducted on the plants that are traditionally used as drugs, and their effects on weight loss and blood glucose control in diabetes patients have been investigated (Mahmood, 2016).

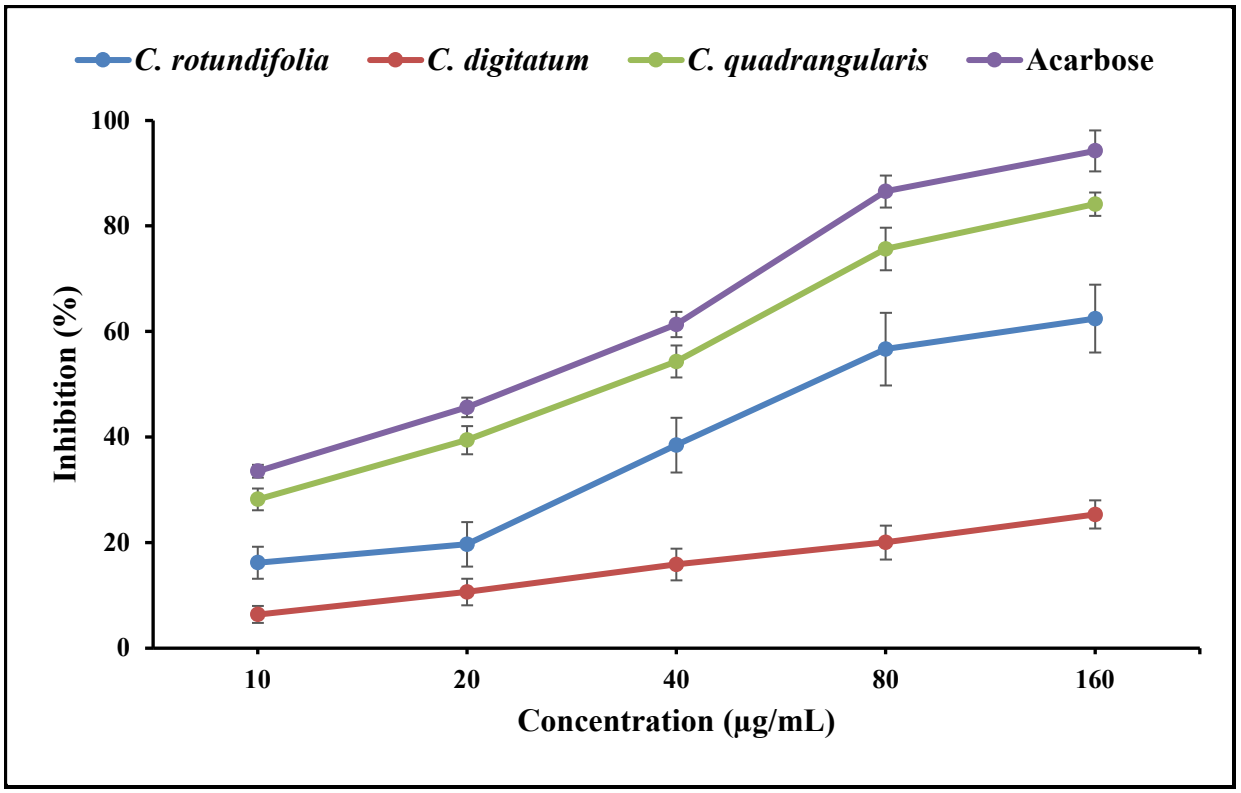

Fig. 2 (A): Alpha-amylase inhibitory activity of methanol extract from the leaves of the studied plants. Data are given as mean ± SD (n = 3).

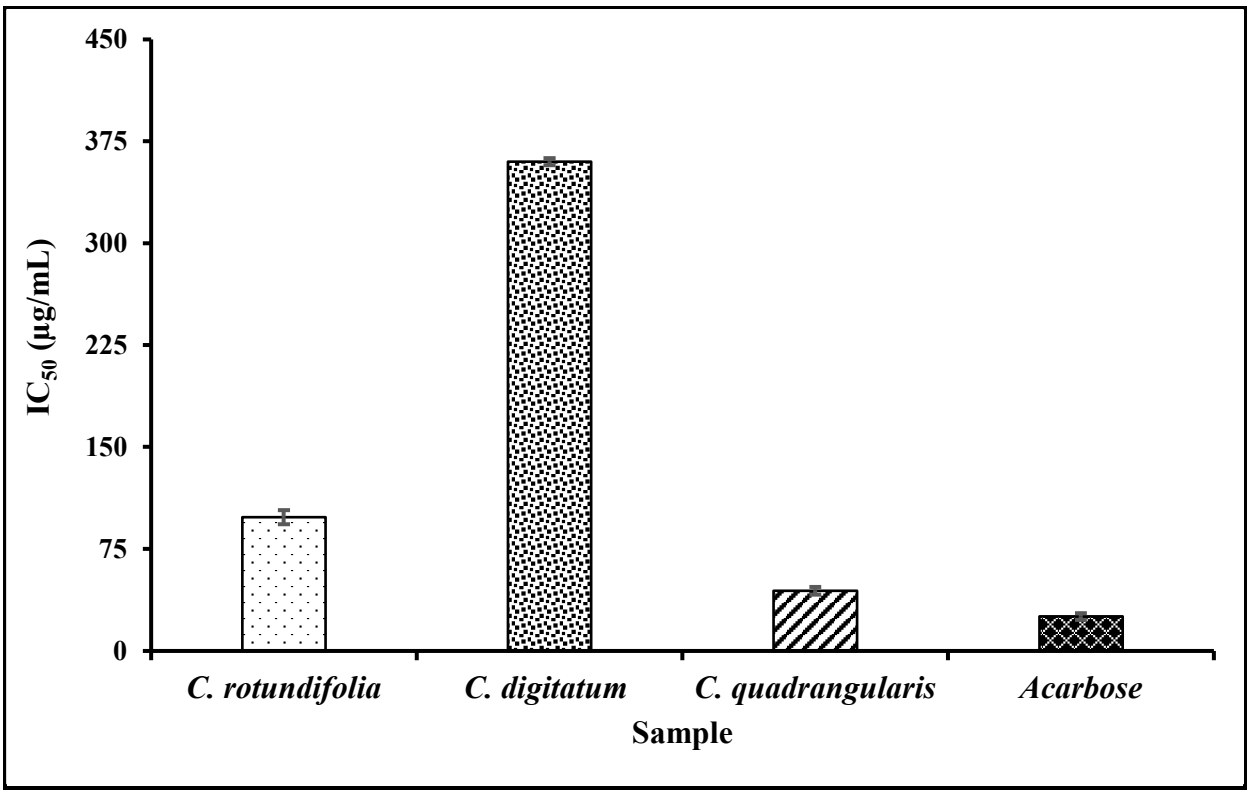

Fig. 2 (B): Half-maximal inhibitory concentration values (IC_{50}) of plant samples and acarbose as a reference inhibitor on alpha-amylase.

Conclusion: The leaves of *C. rotundifolia, C. digitatum*, and *C. quadrangularis* were biochemically characterized, including proximate analysis, nutrients quantity, anti-nutritional factors, bioactive compounds and their antioxidant and anti-diabetic properties. The results showed that the traditional vegetable plants under study could be a potential source of beneficial health components. This suggests that *C. rotundifolia* leaves may serve as a good source of various nutrients and as a promising source of affordable plant proteins. The levels of the heavy metals detected in the tested extracts were below the permissible limit for vegetable plants. *C. rotundifolia* and *C. digitatum* leaves are the richest samples of vitamin C and provitamin A and can be good sources of these micronutrients. The highest amounts of the biologically active component were found in *C. quadrangularis* extract. The obtained results prove that *C. rotundifolia* possessed essential nutritive values, and antioxidant agents, while *C. quadrangularis* had useful biological properties. The inhibitory activity of the alpha-amylase enzyme was increased with the increase in concentration of the extract. *C. quadrangularis* leaves inhibited alpha-amylase *in vitro* with inhibitory activity comparable to acarbose, a reference anti-diabetic agent. Overall, these findings suggest a need to bring back the use of these wild vegetables in a mixed diet because they have several benefits.

Conflict of interest: The authors declare no conflict of interest regarding this study.

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