Asian Journal of Chemistry; Vol. 33, No. 2 (2021), 376-380

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.22882

# *in vitro* Determination of Nutritive and Antinutritive Contents of *Helichrysum petiolare* Hilliard & B.L. Burtt

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*Received*: 3 July 2020; *Accepted*: 23 November 2020; *Published online*: 15 January 2021; AJC-20224

Till date, less studies have been done on the nutritive and antinutritive constituents of *Helichrysum petiolare*. This study therefore evaluated the nutritive and antinutritive constituents of the plant using proximate analysis and standard laboratory procedures, respectively. The result showed high levels of acid detergent fibre (ADF), vitamins (A, C and E), neutral detergent fibre (NDF) and minerals in *H. petiolare*. The high ADF level was believed to be responsible for the low energy, fat and carbohydrate levels observed in this study. The result also showed high level of oxalate and therefore, suggests cooking of the plant before human consumption. Overall nutrition, antinutritional and mineral compositions of the plant showed that the *H. petiolare* is immensely rich in vital nutrients that are of great importance to health and metabolism. These nutrients are suggested to be partly responsible for the plant's useful medicinal properties.

**Keywords: *Helichrysum petiolare*, Diabetes, Proximate analysis, Acid detergent fibre, Neutral detergent fibre, Oxalate.**

## INTRODUCTION

Medicinal plants have long been employed in traditional medicine for the treatment of many illnesses in Africa and all over the world [1]. Traditional medicine is defined by the World Health Organisation (WHO) as health practices and beliefs that employ the use of animal, mineral and plant-based medicines to prevent, diagnose and treat diseases. Plants are available in various species, and as a result, their use has become more widely accepted in traditional medicine. Furthermore, in Africa, about 80% of the population use traditional medicine to meet essential health care needs and is vital in the battle against illnesses [2]. More than 1,123 plant species are used worldwide and in South Africa for ethnopharmacological or experimental treatment of several diseases like diabetes [2,3].

According to Erasto *et al*. [4], *Helichrysum petiolare* a shrub of the Asteraceae family possesses strong hypoglycaemic properties. Helichrysum species is one of the most important shrubs in South Africa in terms of culture, medicine and history, and they have not enjoyed horticultural exposure [5]. *Helichrysum petiolare* spreads from the Eastern Cape (old Transkei), *via* Drakensberg and Lesotho to the tropical Africa [6]. Because it rises on high altitudes, it is frost or drought-tolerant. The leaves are commonly cooked and eaten, and the roots and leaves are commonly used as standard therapeutics for diabetes colic, mellitus, fever, colds, inner sores, coughs, chest complaints, nausea , fatigue and dressing wounds [7].

The medicinal properties of *H. petiolare* are suspected to be partly due to its nutritional content [8]. Nutrition is especially important when a vegetable or plant is used as a food source. All vegetables contain minerals, vitamins and proteins, which are nutritious for humans' and animals' growth and development. The leaves are also sources of carbohydrates and pigments in foods [9]. It is vital to note that the nutrients present may be influenced by endogenous toxic factors within the plant or vegetable. These factors are referred to as anti-nutrients which limit the effective use of the plant's nutrient by binding to digestive enzymes and some dietary proteins. Consequently, they reduce the ability of the plant's nutrients, such as protein, minerals and vitamins, and in turn impair the nutritional benefit of such nutrient [10]. Oxalic acid, tannins, phytic acid and hydrocyanic acid are the most common anti-nutritional factors in leafy vegetables [11].

To date, less research has been made into the nutritional and anti-nutritional constituents of *Helichrysum petiolare*, most importantly into its micro and macro nutritional contents. This

study, therefore, reported the nutritional and anti-nutritional potentials of *H. petiolare*.

## EXPERIMENTAL

**Plant collection:** The whole plant of *H. petiolare* was collected from Hogsback, in Raymond Mhlaba Municipality of Eastern Cape, South Africa. The plant was identified and authenticated by Prof. C.N. Cupido, Department of Botany, University of Fort Hare, Alice, South Africa, and a voucher was submitted at the Giffen herbarium, University of Fort Hare, Alice Campus, Eastern Cape, South Africa.

**Plant preparation:** The whole plant was washed, oven-dried (40 ºC), and pulverized using an electrical blender. The pulverized sample was then passed through the 20 µ mesh sieve, and refrigerated at 4 ºC until further use.

### Proximate analysis

**Determination of moisture content:** The moisture content was determined as described previously [12]. Briefly, a sterile crucible dried to a constant weight at 110 ºC in an air oven was cooled in a desiccator and weighed ($W_1$). Two grams of finely ground fresh sample were then carefully weighed into the labelled crucible and reweighed ($W_2$). The crucible holding the sample was dried to a steady weight in the oven ($W_3$) and the percentage (%) moisture content was determined using eqn. 1:

$$\text{Moisture content } (\%) = \left(\frac{W_2 - W_3}{W_2 - W_1}\right) \times 100$$

**Determination of ash content:** The ash content of the plant was determined using the method described previously [12]. Dried porcelain crucible already labelled with a heat resistant marker was weighed ($W_1$). About 1 g of the pulverized plant sample was placed in the preweighed porcelain crucible and reweighed ($W_2$). The crucible now containing the plant sample was arranged into the muffle furnace programmed to ash initially at 250 ºC for 1 h and further at 550 ºC for 5 h. The crucible containing the ash was removed after complete ashing, cooled in a desiccator and weighed ($W_3$). The percentage of ash was calculated using eqn. 2:

$$\text{Ash content } (\%) = \left(\frac{W_3 - W_1}{W_2 - W_1}\right) \times 100$$

**Crude fat content determination:** The crude fat content of the plant was also determined as described previously [12]. The weighed powdered sample (5 g) was added to a 100 mL of diethyl ether, the flask was covered with aluminium foil and shaken in an orbital shaker for 24 h. The mixture was then filtered and the supernatant ether extract collected differently into a previously weighed ($W_1$) clean beaker. An additional 100 mL diethyl ether was introduced and the mixture was shaken for another 24 h. The filtrate was decanted into the same beaker ($W_1$). The ether (filtrate) was concentrated in a water bath and heated to full dryness in an oven at 40-60 ºC. The beaker was reweighed ($W_2$) and the crude fat content was calculated using eqn. 3:

$$\text{Crude fat } (\%) = \left(\frac{W_2 - W_1}{\text{Weight of the original sample}}\right) \times 100$$

**Determination of neutral detergent fibre (NDF):** Air-dried whole plant sample of *H. petiolare* was ground to pass through 1 mm seive. At room temperature, 0.5 g sodium sulphite and a few drops of *n*-octanol were placed in the crucible containing 1 g of plant sample and 100 mL of neutral detergent solution. The mixture was boiled and allowed to reflux for 60 min from after which it was filtered, washed three times with boiling water and then twice with cold acetone. The residue was dried at 105 ºC for 8 h and left to cool in the desiccator. The dried residue was weighed and the neutral detergent fibre was calculated using eqn. 4:

$$\text{NDF } (\%) = \left(\text{Weight of crucible} + \text{Weight of residue}\right) - \left(\frac{\text{Weight of crucible}}{\text{Weight of sample}}\right) \times 100$$

**Determination of acid detergent fibre (ADF) :** Air-dried whole plant sample of *H. petiolare* was ground to pass 1 mm seive. At room temperature, a few drops of *n*-octanol were poured into the crucible now containing 1 g of pulverized sample and 100 mL of neutral detergent solution. The mixture was boiled and allowed to reflux for 60 min. After which, it was filtered, washed three times with boiling water and then twice with cold acetone. The residue was dried at 105 ºC for 8 h and left to cool in the desiccator. The dried residue was weighed and the acid detergent fibre was calculated using eqn. 5:

$$\text{ADF } (\%) = \left(\text{Weight of crucible} + \text{Weight of residue}\right) - \left(\frac{\text{Weight of crucible}}{\text{Weight of sample}}\right) \times 100$$

**Determination of crude protein:** Pulverized plant sample (2 g) was digested in a Kjeldahl flask by boiling in 20 mL of conc. $H_2SO_4$ and a digestion tablet (catalyst) until the mixture was clear. The digest was filtered, marked up to 250 mL and then distilled. An aliquot (50 mL of 45% NaOH solution) was transferred into a 500 mL round bottom flask and distilled. Plant distillate (150 mL) was collected into a flask containing 100 mL of 0.1 N HCl. This was then titrated against 2 M NaOH using methyl orange as an indicator to give a colour change of yellow (endpoint). The % nitrogen content was calculated using eqn. 6:

$$\frac{\left[(\text{mL standard acid} \times N \text{ of acid}) - (\text{mL blank} \times N \text{ of base}) - (\text{mL standard base} \times N \text{ of base}) \times 1.4007\right]}{\text{Weight of sample (g)}}$$

where N is normality.

The amount of crude protein was estimated as the product of a constant factor (6.25) and the quantity of nitrogen. The percentage of crude protein in the sample is nitrogen × 6.25.

**Total non-structural carbohydrate (TNC) content determination:** The TNC content was determined using the method described by Gent [13]. Pulverized whole plant sample (30 mg) was rehydrated and digested for 42 h at 37 ºC with 5 mg/mL

α-amylase to solubilize the carbohydrates. The solubilized carbohydrates were hydrolyzed and its inherent reducing sugars were calorimetrically measured by reduction of $K_3Fe(CN)_6$. This procedure calculated TNC in grams of glucose equivalent per gram of dry weight. The study was replicated up to five times in order to reduce the standard error below 5 mg/g TNC for each sample.

**Caloric content determination:** The calorie values were estimated by summing up the multiplied values for crude protein, crude fat and carbohydrate using factors (4 kcal, 9 kcal and 4 kcal), respectively. The energy ratio of protein (PEP), total fat (PEF) and carbohydrate (PEC) as PEP%, PEF% and PEC% is thus determined accordingly:

$$\text{Energy value (kcal/100 g)} = (\text{crude protein} \times 4) + (\text{crude fat} \times 9) + (\text{total carbohydrate} \times 4)$$

All the analyses were done in triplicate.

### Vitamin analysis

**Vitamin C content determination:** Sample (1 g) was soaked with 20 mL of 0.4% oxalic acid and filtered with Whatman No. 1 filter paper. Thereafter, 9 mL of indophenol reagent was added to 1 mL of the filtrate and the absorbance was measured at 520 nm. The vitamin C content was extrapolated from a vitamin C standard curve. The vitamin A content of the sample was determined as retinol equivalent in mg/100g, from the standard curve by the equation: $Y = 2.6707x + 0.585$, $R^2 = 0.928$.

**Vitamin A content determination:** In brief, ground material (1 g) was macerated with 20 mL of petroleum ether. It was decanted into a test tube and then evaporated to dryness. Approximately 0.2 mL of chloroform-acetic anhydride (1:1 v/v) and 2 mL of trichloroacetic acid-chloroform (1:1 v/v) were added to the residue and the absorbance was taken at 620 nm. The standard for vitamin A was prepared in a similar way and the absorption was measured at 620 nm. The vitamin A concentration in the sample was extrapolated using the standard curve equation: $y = 1.0318x + 0.0013$, $R^2 = 0.9972$, where y is the absorbance and $x$ is the concentration of vitamin A.

**Vitamin E content determination:** Briefly, 1 g of plant sample was macerated with 20 mL of ethanol and then filtered. Ferrous chloride (0.2%) dissolved in ethanol was added to 1 mL of the filtrate, and the mixture was then diluted with distilled water to 5 mL. Absorbance was taken at 520 nm. The standard solutions were prepared using similar method and vitamin E concentration was extrapolated using the calibration curve equation from the standard curve: $y = 12.537x + 0.4146$, $R^2 = 0.9251$, where y is the absorbance and $x$ is the concentration of vitamin E. All the experiments were done in triplicates.

### Determination of macro- and microminerals

**Digestion of plant sample:** A digestion mixture made up of sulphuric acid, selenium powder and salicylate was prepared. Approximately 0.3 g of pulverized plant material was placed in dry, clean digestion tubes. In each tube a volume of 2.5 mL of the digestive mixture was added and the solution was left to react for 2 h at room temperature. The tubes were heated for 1 h at 110 ºC in a block digester and then left to cool. Due to the volatility of the reaction three successive portions of 1 mL hydrogen peroxide were added at intervals of 10 s. The tubes were again further digested at 330 ºC until their contents turned from coloured to clear.

**Analysis of digested samples:** The digested samples were cooled to room temperature, transferred to 50 mL volumetric flasks and filled to the mark with deionized water. Standards were developed for all individual elements to be analyzed. Determination of the microminerals (iron, zinc, manganese and copper) and macrominerals (calcium, magnesium, potassium, sodium and phosphorus) contents were done using inductively coupled plasma-optical emission spectrometer (ICP-OES; SMM Instruments, SUD Africa Variary 710-ES serial) [14]. All tests were performed in triplicates.

### Anti-nutrient analysis

**Oxalate content determination:** Oxalate was determined by using the method described by Essack *et al.* [15]. Briefly, 190 mL of distilled water and 10 mL of 6 M HCl was applied to 1 g of the sample in a 250 mL volumetric flask. The mixture was then heated at 90 ºC for 4 h in a water bath and the digested sample centrifuged for 5 min at 2000 rpm. The supernatant was diluted to 250 mL, and its three 50 mL aliquots were evaporated to 25 mL. The brown precipitate was filtered and washed. The mixed solution was then titrated in droplets with concentrated ammonia solution until the pink methyl orange colour changed to yellow. The solution was then heated to 90 ºC and oxalate was precipitated with a solution of 5% calcium chloride and left to stand overnight. The solution was centrifuged and the precipitate was diluted to 125 mL after it has been washed with 25% hot sulphuric acid. It was then titrated against 0.05 M $KMnO_4$, and the amounts of oxalate was estimated using formula:

$$1 \text{ mL } 0.05 \text{ M } KMnO_4 = 2.2 \text{ mg Oxalate}$$

**Phytic acid content determination:** Phytic acid was determined using the method described by Kibiti *et al.* [16]. Briefly, weighed sample (2 g) was soaked in 100 mL of 2% conc. HCl for 3 h and then filtered with a Whatman No. 1 filter paper. To 50 mL of the filtrate, 10 mL of distilled water were added to give proper acidity. Then, to this solution, 10 mL of 0.3% ammonium thiocyanate solution was added and titrated with standard $FeCl_3$ solution containing 0.00195 g Fe/mL, the observed endpoint was yellow which persists for 5 min. The percentage of phytic acid was calculated as:

$$\text{Phytic acid (\%)} = y \times 1.19 \times 100$$

where y = titre value × 0.00195 g.

The phyto-nutrient, vitamins, macro- and micro-elements contents were expressed as mg/100 g.

## RESULTS AND DISCUSSION

*Helichrysum petiolare* exhibits medicinal properties. It is in fact, one of the best known and commonly used herbal medicines in South Africa. Table-1 shows the proximate composition of *H. petiolare*. According to the result, neutral detergent fibre (NDF) had the highest value (68.55 ± 0.55%), followed by acid detergent fibre (ADF) (51.72 ± 3.05%).

The NDF value observed in this study is the total cell wall, which comprises of the ADF fraction and hemicellulose. NDF

TABLE-1
PROXIMATE COMPOSITION (%) OF *H. petiolare*

| Parameters | Dry matter |
|---|---|
| Moisture content | 12.43 ± 4.43 (%) |
| Ash content | 6.76 ± 0.29 (%) |
| Acid detergent fibre | 51.72 ± 3.05 (%) |
| Neutral detergent fibre | 68.55 ± 0.55 (%) |
| Total non-structural carbohydrate | 1.27 ± 0.47 (%) |
| Protein | 7.17 ± 0.22 (%) |
| Fat | 2.6 ± 0.1 (%) |
| Total energy | 23.92 ± 0.79 (kJ/100 g) |

Data are presented as means ± SD of three replicates.

values are good indicators of bulk and reflect the amount of forage that can be consumed, which are very important [17]. Increase in % NDF is inversely proportional to intake of dry matter,and increases the bulk of stool and the speed of passage of food through the digestive system [18]. Also, increase in NDF creates more balance in the intestinal pH and increased stimulation of short-chain fatty acids production through intestinal fermentation [18]. The high NDF observed in this study, therefore, showed the medicinal potentials of *H. petiolare* in the maintenance of GIT health, treatment of constipation, enhancement of bowel movement, gastric emptying and reduced risk of colorectal cancers.

The ADF value refers to the cell wall portions of the plant that are made up of cellulose and lignin. ADF is important because its value relates to the ability of an animal to digest the plant [19]. As ADF increases, the digestibility of the forage decreases. High ADF values mean lower energy value and decreased digestibility, this explains the low energy, fat and carbohydrate values obtained in this study, and it is in agreement with the work done by Espinosa *et al*. [17], in which they concluded that the plant's ADF value has an inverse effect on digestible energy (DE) and metabolizable energy (ME). High ADF level has also been indicated to aid the gastrointestinal tract (GIT) in the digestion of food and absorption of nutrients from food [18,20]. The high ADF value observed in this study, suggest that the *H. petiolare* may be used in the regulation of serum glucose levels, hence, be useful in the treatment of diabetes, GIT disorders, indigestion and malabsorption. The nutritional analysis of the whole plant of *H. petiolare* also showed high moisture and protein contents in the plant (Table-1). This suggests that the plant is an important source of plant proteins.

Antinutrient assay showed a high level of oxalic acid and a low level of phytic acid in *H. petiolare* (Table-2). This is similar to the work done by Essack *et al*. [15], in which the Asteraceae plants *Bidens pilosa* and *Galinsoga parviflora*, were also found to possess high levels of oxalate (> 85 mg/mL but < 100 mg/mL).

TABLE-2
ANTI-NUTRIENT CONTENT (mg/100 g) OF *H. petiolare*

| Anti-nutrients | Composition (mg/100 g) |
|---|---|
| Phytic acid | 0.37 ± 0.0027 |
| Oxalic acid | 36.23 ± 0.13 |

Data are presented as means ± SD of three replicates.

Antinutrients are natural or synthetic compounds which may interact with nutrient absorption [21]. Oxalic acid can occur as potassium or sodium salt (both soluble) (absorbable), or as insoluble calcium, iron or magnesium salts (non-absorbable) [15]. The absorbable soluble salt can form strong chelates with dietary calcium, reducing its bioavailability and also inhibiting its absorption by the body. Also when consumed in high concentration, the soluble oxalate can form kidney stones [15]. Diets high in oxalic acid, therefore, may need supplementation of minerals to avoid deficiency [9]. Since there is a moderately high level of oxalate in uncooked *H. petiolare*, its high consumption may result in the formation of kidney stones and/or impaired calcium absorption from the small intestine.

Vitamins are essential micronutrients needed by an organism in small quantities for proper metabolism [22]. The results showed *H. petiolare* contains a high amount of vitamins C and E, and very high level of vitamin A (Table-3), this is similar to the work done by Bouba *et al*. [23] in which *Echinops giganteus*, a member of the Asteraceae family was also shown to have a similar level of vitamin A four to five times higher than the levels of vitamins C and E. The result showed a high level of vitamin C in *H. petiolare* and justifies the traditional use of the plant in the treatment of injuries and prevention of wound infections, as vitamin C is highly effective and required in wound healing and tissue regeneration, while vitamins A, C, and E as antioxidants can scavenge free radicals produced in tissue inflammation as a result of injuries and several metabolic diseases such as diabetes.

TABLE-3
VITAMIN CONTENT (mg/g) OF *H. Petiolare*

| Vitamin | Composition (mg/g) |
|---|---|
| Vitamin A | 40.55 ± 0.30 |
| Vitamin C | 6.22 ± 0.11 |
| Vitamin E | 3.50 ± 0.065 |

Data are presented as means ± SD of three replicates.

Several minerals (*e.g*. Ca, Mn, Zn, *etc*.) found in some medicinal plants are co-factors to key enzymes of insulin action and glucose metabolism. Table-4 shows the levels of macro- and micro-minerals in *H. petiolare*. According to the result, there are very high levels of iron and manganese and moderately high levels of copper and zinc in the plant. *Echinops giganteus* a member of the Asteraceae family, was also found to have similarly high levels of iron and manganese [23].

High level of manganese was recorded in this study, therefore, it gives more explanation on the high free radicals scaven-

TABLE-4
MACRO- AND MICROELEMENTS
COMPOSITION (mg/100 g) OF *H. petiolare*

| Mineral element | Composition (%) | Mineral element | Composition (%) |
|---|---|---|---|
| Calcium | 0.68 ± 0.071 | Phosphorus | 0.045 ± 0.0071 |
| Copper | 15 ± 2.83 | Potassium | 1.28 ± 0.071 |
| Iron | 308.5 ± 23.33 | Sodium | 0.43 ± 0.028 |
| Magnesium | 0.205 ± 0.02 | Zinc | 56.5 ± 3.54 |
| Manganese | 605 ± 79.20 | K/Ca + Mg (%) | 0.645 ± 0.035 |

Data are presented as means ± SD of three replicates.

ging abilities of *H. petiolare* reported by previous studies [24,25], and also confirms the usefulness of the plant in the treatment of several diseases and infections. *H. petiolare* was high in zinc, it may, therefore, be useful in the prevention or treatment of clinical conditions that may arise as a result of zinc deficiency. Moreover, *H. petiolare* is also rich in the other minerals too, therefore, it may be useful in the prevention and treatment of copper deficiency and its accompanying health conditions. The results also showed a very high level of iron in *H. petiolare*.

## Conclusion

*Helichrysum petiolare* is a potent herbal medicine popularly used in the Eastern Cape of South Africa in the herbal treatment of diabetes, infections and other diseases. This study successfully sheds more light on the constituent minerals and nutrients of the plant. The presence and levels of these minerals and nutrients must have contributed greatly to the plant′s numerous useful medicinal properties. Methods of processing can have a reductive effect on the levels of the plant′s micronutrients. Thermal processing, for example, enhances the bioavailability of vitamin A but reduces that of vitamin C phytates reduce the bioavailabilities of plant′s iron and zinc, while the minerals′ efficacies are enhanced by the presence of vitamin C and protein. The low phytic acid and high vitamin C that were observed in this study, therefore, explain and justify the high levels of iron and zinc recorded. More research, however, need to be done on the antinutrient and mineral constituents of the acetone, ethanol, and cold and boiled aqueous extracts of *Helichrysum petiolare*, and the effects of those constituents on the extracts′ medicinal properties.

## ACKNOWLEDGEMENTS

The authors thank the National Research Foundation (NRF) of South Africa for funding this work.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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