

Phytochemical Screening and *In-Vitro* Antioxidant Activities of Various Extracts of *Helichrysum petiolare* Hilliard & B.L. Burtt used for the Treatment of Diabetes Mellitus in the Eastern Cape Province of South Africa

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Abstract: The phytochemical contents of the acetone, ethanol, and boiled and cold aqueous whole-plant extracts of *Helichrysum petiolare* were determined using standard phytochemical reaction methods. ABTS, DPPH, NO and TAC assays were used to evaluate their antioxidant properties. This study reported the highest total phenolic content (212,963 mg/g) in the boiled aqueous extract, while the ethanol had the highest flavonoid (172.393 mg/g) and proanthocyanidin contents (65.855 mg/g). Alkaloids, flavonols, and saponin were highest in the acetone extract, while the cold aqueous extract had the lowest phytochemical content. Among the extracts, the boiled aqueous extract had the highest DPPH^{••} (IC₅₀ 0.02 mg/mL) and ABTS^{••} (IC₅₀ 0.07) inhibition capacities, while the ethanol extract exhibited the highest NO[•] inhibition (IC₅₀ 0.41 mg/mL) and TAC (IC₅₀ 0.19 mg/mL). These findings justify the use of *H. petiolare* in traditional medicine and further recommend the ethanol and boiled aqueous extracts of the plant as more effective extracts for medicinal treatment.

Keywords: ABTS, Alkaloid, DPPH, *Helichrysum petiolare*, Proanthocyanidin, Saponin.

1. INTRODUCTION

Phytochemicals are synthesized by plants through primary or secondary metabolism [1] and play several vital roles in plants, which include defence against predators, competitors or pathogens, plant growth, etc. [2,3]. Some phytochemicals are toxic to humans (phytotoxins) [4]; these include sanguinarine which at low doses can cause cancer [5]. Some have anti-nutrient properties and limit the absorption of nutrients [6], while others, like flavonoids and polyphenols, might be pro-oxidants when ingested in high amounts [7]. Roughly 10,000 different phytochemicals have been identified, while many are still unknown. A few phytochemicals such as terpenes, flavonoids, isoflavones, indoles, phytic acid, glucosinolates, polyphenols, isothiocyanates, and carotenoids, however, have antioxidative and medicinal benefits.

Antioxidants are compounds that inhibit free radical-induced oxidation. Some antioxidants like ascorbic acid (vitamin C) or thiols terminate the chain reactions in free radical generation/oxidation. Complex systems of overlapping antioxidants are maintained by plants

and animals in their quest to balance the oxidative state. In animals, these antioxidants are either produced internally e.g. glutathione, superoxide dismutase and catalase or derived from the diet e.g. vitamins E and C [8]. Antioxidants operate at different levels by scavenging free radicals, inhibiting the formation of ROS, or increasing the capabilities of antioxidant enzymes. Endothelial dysfunction in type 2 diabetes mellitus (T2DM), for example, could be potentially improved by supplementation with antioxidants and/or factors essential to nitric oxide (NO) production by re-coupling mitochondrial function and endothelial nitric oxide synthase (eNOS), as well as decreasing vascular NAD(P)H oxidase activity [8].

Oxidative stress is any alteration in the balance of the body's antioxidants and free radicals in favour of the free radicals, caused by factors like drug actions, addiction, toxicity, ageing and inflammation [9]. It is in general, defined as the increased systemic manifestation or/and inadequate removal of reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive chlorine species (RCS) [10]. In a metabolic disease like diabetes particularly type 2 diabetes, oxidative stress is believed to play a key role in vascular complication development [11]. Tissues are made susceptible to oxidative stress as a result of variation in the levels of antioxidant enzymes, which leads to the development of diabetic complications [12].

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The concept that diets high in fruits and vegetables reduce the risk of coronary heart disease (CHD), hypertension, stroke and other diseases evidenced by dose-response relationships has been supported by critical reviews of studies available in the literature [13]. The critical role played by phytochemicals in relieving the body of oxidative stress and reducing the risk of several diseases such as cancer and inflammatory conditions have also been confirmed by several research groups [14]. Recent studies, for example, has cited various effects of phytochemical consumption on reduction in stroke risk [15], cancer prevention [16], and type 2 diabetes prevention [17]. Mechanisms of action proposed for these findings include inhibition of lipid-lowering effects, anti-inflammatory activity, lipid oxidation, antioxidant activity, anti-proliferative or apoptotic cell death activity, as well as hypoglycaemic- and insulin-lowering effects [17].

Plants have copious amounts of natural antioxidants and phytochemicals like polyphenol and various antioxidative compounds which adsorb and neutralize reactive oxygen species [18].

Asteraceae families are well-known sources of antioxidants and antimicrobial agents [19]. The leaves and roots extracts of *Helichrysum petiolare* have been reported to possess antihypertensive and anti-diabetic effects [20]. Previous studies on several other member plants of the Asteraceae family have reported high antioxidant and free radical scavenging abilities [21]. The objective of this study, therefore, is to evaluate the phytochemical contents and antioxidant capacities of the various extracts of the whole plant of *H. petiolare* a less studied member of the Asteraceae family.

2. MATERIALS AND METHODS

2.1. Sample Collection

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

2.2. Preparation of Extracts

The whole plant was washed, cleaned and oven-dried at 40 °C. The dried sample was pulverized using an electrical blender and sieved (20 µ mesh). A portion

(200 g) of the sample was then soaked individually in 1 L of ethanol, acetone, and water (for cold aqueous extracts) and shaken on an orbital shaker for 24 h, while another portion was boiled in 1 L of water (for boiled aqueous extract) for 15 minutes. The solution obtained was then filtered using a Buchner funnel and Whatman No. 1 filter papers and concentrated at 78 °C and 57 °C respectively for ethanol and acetone extracts using a Rotary vacuum evaporator (Scietek, MODEL: RE 300), while the aqueous extracts were concentrated using a freeze drier. The concentrated extracts were stored at 4 °C in the refrigerator until needed for use [22].

2.3. Phytochemical content analysis of the ethanol, acetone and aqueous plant extracts

i. Total Phenols Determination

The modified Folin-Ciocalteu method as described by Bouaziz-Ketata *et al.* (2015) was used to determine the extracts' total phenolic content. 5 mL of Folin-Ciocalteu reagent in distilled water (1:10 v/v) and 4 mL (75 g/L) of sodium carbonate were mixed with an aliquot of 0.5 mL of each extract (1 mg/mL). The resulting mixtures were then vortexed for 15 s and left to stand for 30 min at 40 °C to develop colour. Absorbance was then measured at 765 nm wavelength using the AJI-C03 UV-VIS spectrophotometer. The results were expressed as mg/g tannic acid equivalent using the equation based on the calibration curve: $Y = 4.7783x + 0.0729$; $R^2 = 0.9986$, where x is the absorbance and Y is the tannic acid equivalent.

ii. Determination of Total Flavonoids

Determination of the flavonoid content was done using the method described by Sowunmi and Afolayan, (2015). 0.5 mL of 2% $AlCl_3$ was briefly prepared in ethanol and then added to 0.5 mL of the extracts. The mixture obtained was left to stand for 60 min at room temperature and the absorbance was measured at 420 nm. The extracts were evaluated at a final concentration of 0.1 mg/mL and the results were calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: $Y = 6.5583x + 0.0674$; $R^2 = 0.9995$; where x is the absorbance and y is the quercetin equivalent.

iii. Determination of Total Flavonols

The flavonol content was determined using the method described by Sowunmi and Afolayan, (2015). 2 mL of each plant extract was mixed with 2 mL of 2% $AlCl_3$ prepared in ethanol, 3 mL of sodium acetate solution (50 g/L) was then added. The mixture was

incubated for 150 min at 20 °C. Absorbance was measured at 440 nm. The total flavonol content was calculated as quercetin (mg/g) equivalent, using the following equation based on the calibration curve: $Y=13.537x + 0.0195$; $R^2 = 0.9986$, where x is the absorbance and Y is the quercetin equivalent.

iv. Determination of Proanthocyanidin

Determination of the total proanthocyanidin was done using the method described by Sowunmi and Afolayan, (2015). 3 mL of 4% vanillin/methanol solution and 1.5 mL HCl was mixed with a volume of 0.5 mL of the extract solution. The resulting mixture was vortexed, left to stand for 15 min at room temperature, and the absorbance was read at 500 nm. The total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the calibration curve equation: $Y = 2.9833x + 0.0192$; $R^2 = 0.9916$, where x is the absorbance and Y is the catechin equivalent.

v. Determination of Saponins

This assay was carried out according to the method described by Sowunmi and Afolayan, (2015). Briefly, 200 mL of 20% was mixed on a shaker with 20 g of the plant for 30 min, after which the mixture obtained was heated and stirred in a water bath at 55 °C for 240 min. The mixture was filtered and the residue obtained was re-extracted as described above. The two extracts were combined and further heated on a water bath at 90 °C to reduce the volume to 40 mL, after which it was transferred into a 250 mL separating funnel and extracted twice using 20 mL diethyl ether. The ether layer was discarded, retaining the aqueous layer to which 60 mL of n-butanol was added. The n-butanol extracts were then washed twice using 10 mL of 5% brine solution. This final solution was then concentrated at 87 °C on a water bath, then oven-dried to dryness at 40 °C. The percentage of saponin content was calculated using the formula:

$$\% \text{ saponin} = \frac{\text{final weight of the sample}}{\text{initial weight of the sample}} \times 100$$

vi. Determination of Alkaloids

Alkaloid content was determined according to the method described by Sowunmi and Afolayan, (2015). Briefly, 200 mL of 10% ethanolic acetic acid was mixed with 5 g of the plant extract, covered and left to stand for 240 min. The mixture was filtered, heated in a water bath at 60 °C to one-quarter of its original volume. Concentrated ammonia solution was added to the mixture to trigger precipitation, and then continued until the precipitation stops. The whole solution was then left

for a while to settle, filtered and the precipitate washed with dilute ammonium hydroxide. The residue obtained was dried and weighed and the alkaloid content was calculated using this formula:

$$\% \text{ alkaloid} = \frac{\text{final weight of the sample}}{\text{initial weight of the sample}} \times 100$$

All the experiments were done in triplicates.

2.4. Determination of the Antioxidant Potentials of *H. petiolare*

The antioxidant activities of *H. petiolare* were determined by evaluating the percentage inhibition of free radicals.

i. Total Antioxidant Capacity (TAC)

The TAC of the extracts was determined using the method described by Falode *et al.* (2018). 1 mL of the extract or standard (0.5 – 1.0 mg/mL) solution was pipetted into test tubes at varying concentrations. Thereafter, 3 mL of phosphomolybdate reagent (28 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 M sulphuric acid) was added to each of the test tubes (The blank solution contained 4 mL reagent solution only). The test tubes were capped and incubated in a boiling water bath at 95 °C for 150 min. The samples were left to cool to room temperature, and the absorbance of each solution was measured at 695 nm against blank in a spectrophotometer. The antioxidant capacity was expressed as the rutin equivalent.

ii. Determination of NO Radical (NO[•]) Scavenging Activity

Determination of the NO[•] scavenging activity of the extract was done using the method of Falode *et al.* (2018). 0.5 mL of the extract and standards of varying concentrations (0.2 – 1.0 mg/mL) was added to 2 mL of 10 mM sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) and incubated for 2.5 h at 25 °C. 1 mL was then taken from the incubated mixture and combined with 1 mL of Griess reagent (equal volume of 0.33% Sulphanilic reagent (sulphanilic acid dissolved in 20% glacial acetic acid) and 0.1%(w/v)1-naphthylendiaminedichloride (prepared in distilled water)), this was then incubated at room temperature for 30 min. The absorbance was measured at 540 nm and percentage NO[•] inhibition by the extract calculated using the equation:

$$\text{NO}^\bullet \text{ scavenging activity (\%)} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100$$

Where $Abs_{control}$ was the absorbance of NO^{\bullet} ; Abs_{sample} was the absorbance of NO^{\bullet} + sample or standard.

iii. Determination of Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity

The method described by Falode *et al.* (2018) was used to determine the scavenging activity of DPPH free radical ($DPPH^{\bullet}$) of the extract. A stock solution of 0.135 mM DPPH was prepared in methanol. 0.1 mL of the extract and standards of varying concentrations (0.005 – 0.08 mg/mL) was added to 1 ml of the stock solution. The reaction mixture was then vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using the spectrophotometer. The ability of the plant extract to scavenge $DPPH^{\bullet}$ was calculated from the equation:

$$DPPH^{\bullet} \text{ scavenging activity} = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \times 100$$

Where $Abs_{control}$ is the absorbance of $DPPH^{\bullet}$ + methanol; Abs_{sample} is the absorbance of $DPPH^{\bullet}$ + sample/standards.

iv. Determination of ABTS Radical ($ABTS^{\bullet}$) Scavenging Activity

The ability of the plant extracts to scavenge-2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS$) radical was determined using the methods of Ahmed *et al.* (2015). The working solution was prepared by mixing 2.4 mM of potassium persulfate and 7 mM of $ABTS$ in the ratio 1:1 in distilled water. The mixture was left to react in the dark for 12 h at room temperature. After 12 h, 3 mL of the working solution was further diluted with 150 mL methanol to obtain an absorbance of 0.706 ± 0.002 units at 734 nm using a spectrophotometer. This was adjusted by mixing of $ABTS$ previously prepared using the method outlined above. 1 mL of the working solution was then added to the extracts at varying concentrations (0.2 – 1.0 mg/mL) and allowed to react in the dark. The absorbance was measured at 734 nm after 7 min. The $ABTS^{\bullet}$ scavenging capacity was compared with BHT and ascorbic acid. The percentage of inhibition was calculated as follows:

$$ABTS^{\bullet} \text{ scavenging activity} = \left(1 - \frac{Abs_{sample}}{Abs_{control}} \right) \times 100$$

Where Abs_{sample} is the absorbance of $ABTS^{\bullet}$ + sample (extract or standard)

$Abs_{control}$ is the absorbance of $ABTS^{\bullet}$ + methanol.

3. RESULTS

The phytochemical content of whole plant extracts of *H. petiolare* is shown in Table 1. Based on the results obtained, the boiled aqueous extract ($212,963 \pm 0,260$ mg/g) of *H. petiolare* had the highest overall phenolic content compared to other extracts.

The ethanol extract also had a very high phenolic content, with the highest levels of flavonoids (172.39 ± 5.34 mg/g) and proanthocyanidins (65.86 ± 1.73 mg/g) among the extracts. The acetone extract also had flavonol ($143.87 + 0.55$ mg/g), saponin ($263.73 + 1.60$ mg/g) and alkaloid ($28 + 0.99$ mg/g) contents that were significantly higher than those of the other extracts.

The *In vitro* antioxidant test of the whole plant extracts of *H. petiolare* demonstrated important and concentration-dependent total antioxidant potential (TAC) relative to the standards (i.e. ascorbic acid and BHT) (Figure 1).

Table 2 displays the IC_{50} of the extracts and standards against DPPH, ABTS, and NO radicals. According to this study, the four plant extracts had dose-dependent $DPPH^{\bullet}$ scavenging activities (Figure 2), but their activities were relatively low compared to all the standards at concentrations above 0.02 mg/mL, but they all had $DPPH^{\bullet}$ scavenging abilities that were greater than or equal to ascorbate at concentrations below 0.02 mg/mL. The result also showed significant dose-dependent $ABTS^{\bullet}$ scavenging activity across all extracts (Figure 3), activity was much higher than that found in $DPPH^{\bullet}$, and activity was higher for acetone (IC_{50} 0.02 mg/ml) and boiled aqueous extracts (IC_{50} 0.02 mg/ml) at concentrations above 0.01 mg/mL.

All plant extracts showed elevated inhibition of NO^{\bullet} greater than 80%. The activities of the extracts were extremely competitive with those of the standards. However, the activities of the extracts were non-dose-dependent, except for acetone and ethanol extracts, which showed a slight decrease in activity with an increase in concentration.

4. DISCUSSION

Analysis of acetone, ethanol, cold and boiled aqueous extracts from the entire plant of *H. petiolare* revealed the presence of saponins, alkaloids, flavonoids, flavonols and proanthocyanidins (Table 1). Proanthocyanidins are condensed tannins with varying pharmacological properties [27]. They possess a broad

Table 1: Phytochemical Constituents of *H. petiolare* Whole-Plant Extracts

Phytochemicals	Boiled Aqueous	Cold Aqueous	Acetone	Ethanol
Total Phenols	212.96 ± 0.26 ^a	147.04 ± 0.26 ^d	204.80 ± 1.30 ^b	187.85 ± 0.78 ^c
Flavonoids	21.67 ± 0.95 ^c	11.45 ± 0.19 ^d	102.86 ± 3.24 ^b	172.39 ± 5.34 ^a
Proanthocyanidins	2.28 ± 0.83 ^c	2.06 ± 0.48 ^c	60.27 ± 0.83 ^b	65.86 ± 1.73 ^a
Flavonols	11.93 ± 0.18 ^d	15.06 ± 0.11 ^c	143.87 ± 0.55 ^a	107.78 ± 0.09 ^b
Saponin	206.07 ± 0.29 ^c	71.8 ± 0.50 ^d	263.73 ± 1.60 ^a	211.67 ± 0.76 ^b
Alkaloids	26.07 ± 1.25 ^b	24.13 ± 1.52 ^c	28 ± 0.99 ^a	25.93 ± 0.29 ^b

Values along the same row followed by different superscript are significantly different ($P < 0.05$).

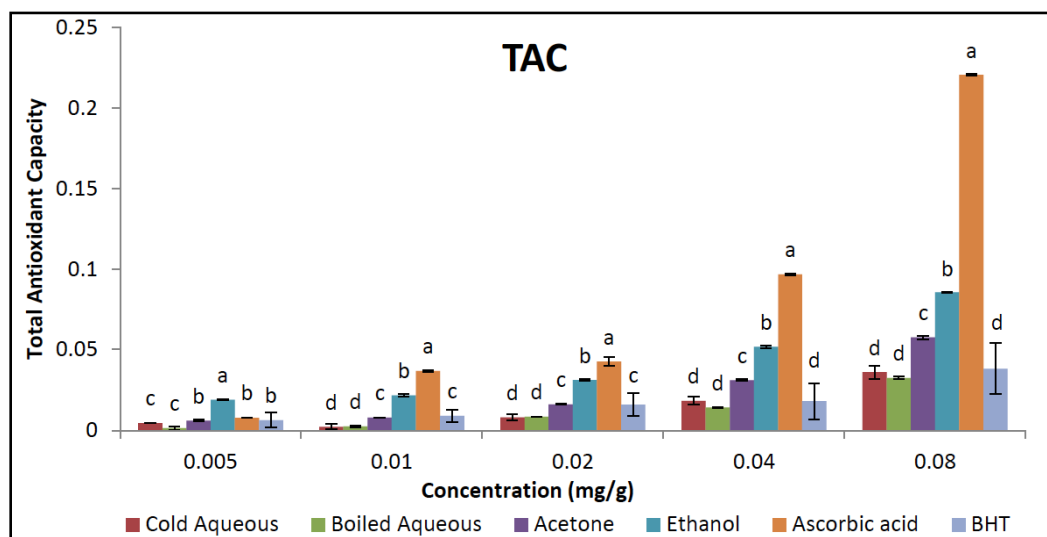


Figure 1: Total antioxidant capacities of ascorbic acid, BHT, cold and boiled aqueous, acetone and ethanol whole plant extracts of *H. petiolare*. Data are presented as means ± SD of three replicates. Points with the same alphabet within the same concentration are not significantly different.

Table 2: Scavenging Activities of Aqueous (Cold and Boiled), Acetone and Ethanol Whole Plant Extracts of *H. petiolare*

Activity	A		B		C		D	
	IC ₅₀ ^a	R ^{2b}	IC ₅₀ ^a	R ^{2b}	IC ₅₀ ^a	R ^{2b}	IC ₅₀ ^a	R ^{2b}
Cold aqueous extract	0.03	89.99	0.12	99.92	0.89	94.04	0.41	98.5
Boiled aqueous extract	0.02	72.47	0.07	99.8	2.74	87.23	0.45	99.34
Acetone extract	0.02	67	0.19	99.94	0.59	99.46	0.26	99.85
Ethanol extract	0.03	92.59	0.18	99.63	0.41	98.23	0.19	99.8
Rutin	0.02	94.76	0.0006	57.75	0.47	86	0.19	99.11
Gallic acid	0.55	78.04	-	-	-	-	-	-
Ascorbic acid	-	-	0.03	79.5	0.52	93.39	0.04	99.96
BHT	0.04	91.65	0.006	61.68	3.93	91.83	0.61	95.23

The letters represent; **A**= ABTS⁺ scavenging activity; **B**= DPPH⁺ scavenging activity; **C**= NO[•] scavenging activity; **D**= TAC; **a**: IC₅₀ is defined as the concentration (mg/mL) sufficient to obtain 50% of a maximum scavenging capacity; **b**: coefficient of determination; values obtained from regression lines with 95% confidence level and -: Values not determined.

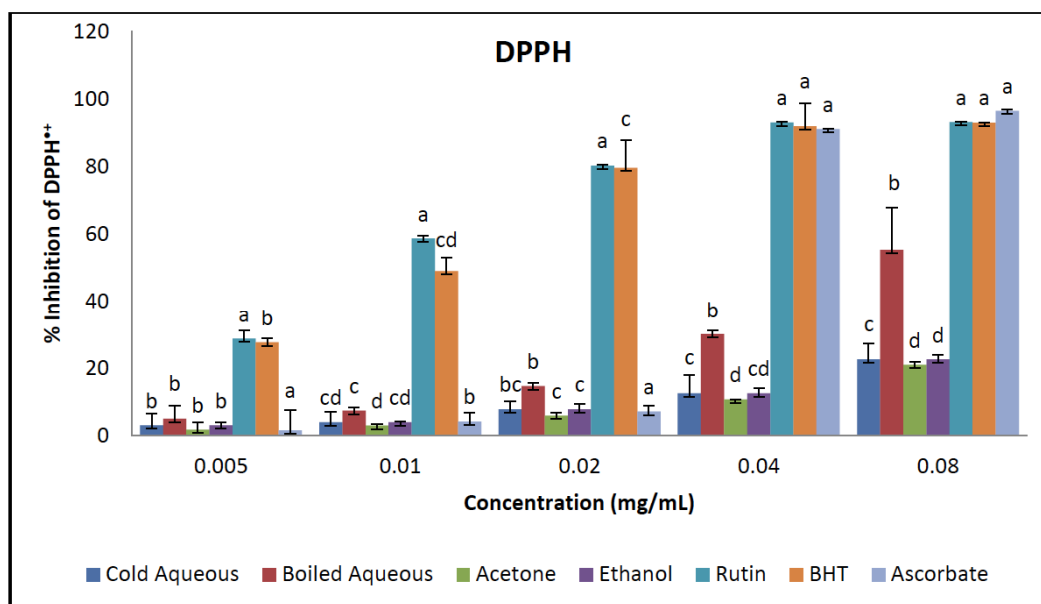


Figure 2: DPPH⁺ scavenging activities of BHT, rutin, ascorbate, ethanol, acetone, boiled aqueous and cold aqueous whole plant extracts of *H. petiolare*. Data are presented as means \pm SD of three replicates. Bar graphs with the same letter superscripts within the same concentration are not significantly different.

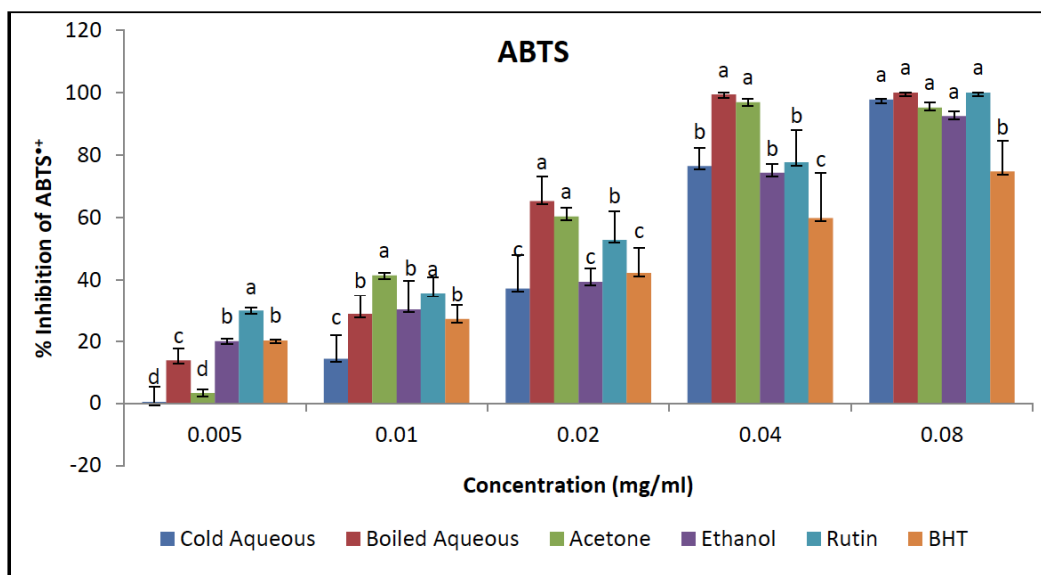


Figure 3: ABTS⁺ scavenging activities of BHT, rutin, ascorbate, ethanol, acetone, boiled aqueous and cold aqueous whole plant extracts of *H. petiolare*. Data are presented as means \pm SD of three replicates. Bar graphs with the same letter superscripts within the same concentration are not significantly different.

variety of beneficial properties including anti-tumour, anti-bacterial, immunostimulating, anti-allergic, anti-oxidant, anti-viral, anti-carcinogenic, anti-inflammatory and vasodilatory properties [27].

Studies have shown that eating proanthocyanidin-rich plants/fruits helps protect the body from sun damage, improve vision, improve joint, artery, and body tissue (e.g heart) flexibility, and improve blood supply by improving the veins, capillaries, and arteries [28]. They can also prevent the accumulation of platelets,

lipid peroxidation and hyperpermeability of capillaries [28,29].

Flavonoids also have a broad variety of biological functions, including antioxidative, analgesic, antiallergic, antiangiogenic, antihypertensive, anticancer, antidiabetic, antimicrobial, and antiinflammatory effects [30]. Their antioxidant effects are also expressed by the inactivation of reactive oxygen species (ROS), thus counteracting plasma low-density lipoprotein (LDL) oxidation and thereby improving blood vessel

endothelium inflammation [31]. Latest findings have demonstrated the health effects of dietary flavonoids, as there was a strong association between their ingestion and lower risk of hypertension and cardiovascular mortality [32]. The very high content of flavonoids and proanthocyanidins in the ethanol extract may be attributable to the high extraction yield of the solvent and explain why the extract has the maximum NO[•] scavenging potential and TAC.

This also confirms the observations of Akinrinde *et al.* (2018) and further supports the utility of *H. petiolare* ethanol extract in the treatment and prevention of hypertension, cancer, fire injuries and other forms of injuries, allergies, common diseases, obesity, insulin resistance, coronary heart disease and diabetes. These findings also illustrate why in conventional herbal therapy, boiled aqueous extracts and ethanol herbal concoctions are preferable, as high phenolic content has been shown to associate strongly with high antioxidant activity by some writers [34], and other studies have put more emphasis on the main role of phenolic compounds as free radical scavengers [35].

Three of the main subclasses of flavonoids (e.g. quercetin, kaempferol, and myricetin) are flavonoids, flavonols, and flavanols or catechins [36], which are mildly ingested in an intestine with normal bacterial flora and are heavily metabolized in humans [37]. It has also been stated that flavonol is a flavonoid with substantially strong antioxidant properties. In previous research, increased activity of erythrocyte superoxide dismutase (an antioxidant enzyme in red blood cells) was correlated with the intake of plants rich in flavonol. Also, flavonol induces an improvement in plasma antioxidant potential (the ability to scavenge free

radicals), a decrease in lymphocyte DNA damage and a decrease in urinary 8-hydroxy-2'-deoxyguanosine (a predictor of oxidative damage) [38]. Flavonol has also been reported to possess anticancer, anticoronary heart disease, and anti-diabetic properties in many studies [39,40].

This explains why the highest TAC was found in the ethanol extract with very high flavonoid and flavonol content (Figure 1). In general, *H. petiolare* acetone and ethanol extracts may also be beneficial in preventing and treating cancer, coronary artery disease and diabetes.

The acetone and ethanol extracts in this study had the highest concentration of saponin, followed by the boiled aqueous extract. Saponins are heat stable, amphiphilic, glycosidic compounds that are naturally found in a wide range of plant foods [41]. A previous study found that saponins increase cardiovascular health due to their ability to lower levels of blood cholesterol and body fat, cholesterol absorption was also said to be hindered by the intake of plants rich in saponins by saponins binding with bile salts [42].

In clinical trials, ginger and ginseng's saponins were shown to decrease total and LDL (bad) cholesterol without altering the levels of HDL (good) cholesterol [42]. Therefore in the prevention and treatment of hypercholesterolemia, hyperlipidemia, high blood pressure, atherosclerosis and cardiovascular diseases (CVDs), acetone, ethanol and boiled aqueous extracts of *H. petiolare* might be very useful.

Despite reports from several authors that phenolic compounds are unstable and lose their antioxidant

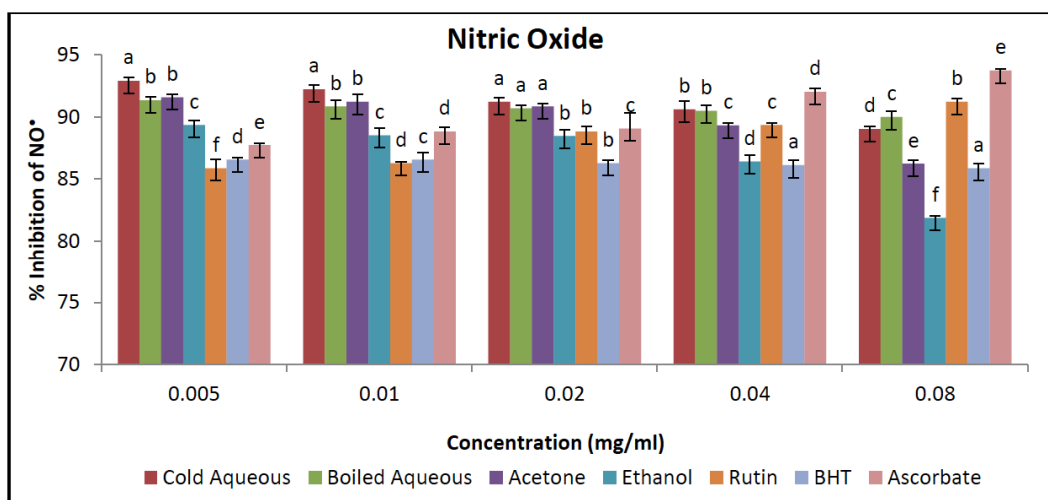


Figure 4: NO[•] scavenging activities of BHT, rutin, ascorbate, ethanol, acetone, boiled aqueous and cold aqueous whole plant extracts of *H. petiolare*. Data are presented as means \pm SD of three replicates. Bar graphs with the same letter superscripts within the same concentration are not significantly different.

capacity once they have been heated [43], the boiled aqueous extract exhibited the highest levels of DPPH⁺ and ABTS⁺ scavenging activity (Figures 2 & 3), this may be due to the high extractive capacity of the decoction method and the presence of heat-stable, viable antioxidant compounds. However, the TAC and NO[•] scavenging capacity of the boiled aqueous extract (Figures 1 & 4) was the lowest; due to heat instability which may have resulted into loss of antioxidant power of the phenols.

The strong ABTS⁺ scavenging potential of all plant extracts compared to rutin and BHT suggests that there were some antioxidant compounds in the plant that can be isolated using water, ethanol and even acetone. Acetone and boiled aqueous extracts exhibited the highest ABTS⁺ scavenging abilities (Figure 3); these high ABTS⁺ scavenging abilities were possibly due to the high saponin and flavonoid content of the extracts. Phenols of boiled aqueous extracts were believed to have grossly reduced antioxidant properties since the previous study found phenols to be unstable by losing their antioxidant properties at high temperatures [43], saponins, however, are more heat stable and show no decrease in antioxidant properties even at high temperatures [41]. High ABTS⁺ scavenging properties of acetone and boiled aqueous extracts, therefore, imply that conditions caused by ABTS⁺-like free radicals may be treated by traditional healers using the plant's acetone and boiled aqueous extracts. The extracts may also be used in the treatment of inflammation, cardiovascular disease, atherosclerosis and hypercholesterolemia.

These observations were similar to the findings of Al-laith *et al.* (2019), in which compounds with high ABTS⁺ scavenging activity reported low DPPH⁺ scavenging activity.

The results also showed that the four plant extracts used in this study had dose-dependent DPPH⁺ scavenging activities (Figure 4), but their activities were quite low compared to all standards at concentrations above 0.02 mg/mL, but they all had DPPH⁺ scavenging potentials greater than or equal to that of ascorbate at concentrations below 0.02 mg/mL. This means that at these low concentrations, the extracts may all be used in replacement of ascorbic acid in cases where there is the scarcity of it. The NO[•] inhibitory activity of acetone, ethanol, boiled and cold aqueous extracts being higher than that of BHT (Table 2) indicates the comparative ability of the extracts to reduce oxidative damage to certain vital tissues in the

body, this is in agreement with several other works done on other species of *Helichrysum* in which similar phytochemicals and antioxidants are present [45,46].

Since NO[•] also plays an important role in the pathogenesis of inflammation [47], *H. petiolare* may, therefore, be very effective for treating hyperglycemia-induced inflammation and wound healing.

5. CONCLUSION

H. petiolare had high phenolic content and strong antioxidant properties. Except for the DPPH⁺ scavenging assay, the ethanol, acetone and boiled and cold aqueous extracts exhibited activities that were higher than BHT and were comparable to ascorbic acid and rutin. This study revealed some of the plant's antioxidant and medicinal potentials. The plant may be used to address the problems of inflammation, atherosclerosis, insulin resistance and cardiovascular disease because it had strong scavenging abilities for ABTS, DPPH and NO-like radicals, which shows that plant extracts may act against oxidation processes in the human body. This may explain its use as a herbal treatment plant in Eastern Cape to treat conditions such as asthma, coughing, pain, colds, infections, chest problems, and high blood pressure. Finally, in addition to the ethanol extract, this study showed that the boiled aqueous extract had more phytochemical content and antioxidant activity than the cold aqueous extract, which means that ethanol and boiled aqueous herbal extracts may be more potent/effective for herbal treatment than the other extracts.

CONFLICTS OF INTEREST

The author declares that there are no conflicts of interest regarding the publication of this paper.

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