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# 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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**Keywords:**

ABTS, Alkaloid, DPPH, Helichrysum petiolare, Proanthocyanidin, Saponin

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**ABSTRACT:** *Helichrysum petiolare* is a herbal plant used in the Eastern Cape of South Africa to treat asthma, chest problems, colds, coughs, infections, diabetes, and high blood pressure. This study aimed to evaluate the phytochemical components and antioxidant activities of the acetone, ethanol, cold, and boiled aqueous extracts of *H. petiolare*. 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 total antioxidant capacity assays were used to evaluate their antioxidant properties. This study showed 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. + ($IC_{50}$ 0.02 mg/mL) and ABTS. + ($IC_{50}$ 0.07) inhibition capacities, while the ethanol extract exhibited the highest NO radical Inhibition ($IC_{50}$ 0.41 mg/mL) and total antioxidant capacity ($IC_{50}$ 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.

**INTRODUCTION:** Phytochemicals are synthesized by plants through primary or secondary metabolism [1] and play several vital roles in plants, which include defense against predators, competitors or pathogens, plant growth, *etc.* [2, 3]

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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, isothiocyanatesols, and carotenoids,

however, have anti-oxidative 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, 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 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 favor of the free radicals, caused by factors like drug actions, addiction, toxicity, aging 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].

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 phyto-chemicals 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, have 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 phyto-chemicals like polyphenol and various anti-oxidative 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]. Therefore, the objective of this study is to evaluate the phytochemical contents and antioxidant capacities of the various extracts of the whole plant of *Helichrysum petiolare,* a less studied member of the Asteraceae family.

## MATERIALS AND METHODS:

**Sample Collection:** The whole plant of *Helichrysum 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.

**Preparation of Extracts:** The whole plant was washed, cleaned, and oven-dried at 40 ºC. 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 min. 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 ºC in the refrigerator until needed for use [22].

**Phytochemical Content Analysis of the Ethanol, Acetone and Aqueous Plant Extracts:**

**1. 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 color. Absorbance was then measured at 765 nm wavelength using the AJI-C03 UV-VIS spectro-photometer.

The results were expressed as mg/g tannic acid equivalent using the equation based on the calibration curve:

$$Y = 4.7783x + 0.0729; R2 = 0.9986$$

Where x is the absorbance and Y is the tannic acid equivalent.

**2. 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:

$$Y = 6.5583x + 0.0674; R2 = 0.9995$$

Where x is the absorbance and y is the quercetin equivalent.

**3. 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 2.5 at 20 ºC. 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:

$$Y=13.537x + 0.0195; R2 = 0.9986,$$

Where x is the absorbance and Y is the quercetin equivalent.

**4. 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:

$$Y = 2.9833x + 0.0192; R2 = 0.9916$$

Where x is the absorbance and Y is the catechin equivalent.

**5. 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 ºC 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 ºC 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 ºC on a water bath, then oven-dried to dryness at 40 ºC. The percentage of saponin content was calculated using the formula:

% saponin = (final weight of sample) / (initial weight of sample) × 100

**6. 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 ºC to one-quarter of its original volume. Concentrated ammonia solution was added to the mixture to trigger precipitation and then continue 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:

% alkaloid = (final weight of sample)/(initial weight of extract) × 100

All the experiments were done in triplicates.

**Determination of the Antioxidant Potentials of Helichrysum Petiolare:** The antioxidant activities of *Helichrysum petiolare* were determined by evaluating the percentage inhibition of free radicals.

**1. Total Antioxidant Capacity:** The total antioxidant capacity 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.

**2. Determination of Nitric Oxide Scavenging Activity:** Determination of the nitric oxide 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 ºC. 1 mL was then taken from the incubated mixture and combined with 1 mL of Griess reagent (equal volume of 0.33% sulphanilic acid and 0.1% (w/v) 46 naphthylene-diaminedichloride prepared in 20% glacial acetic acid), this was then incubated at room temperature for 30 min. The absorbance was measured at 540 nm and percentage nitric oxide inhibition by the extract calculated using the equation:

NO scavenging activity (%) = ((Abs control-Abs sample) / (Abs control)) × 100

Where Abs control was the absorbance of NO radicals; Abs sample was the absorbance of NO radical + sample or standard.

**3. 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 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 radical was calculated from the equation:

DPPH radical scavenging activity = ((Abs control-Abs sample)/(Abs control)) × 100

Where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample/standards.

**4. Determination of ABTS· + 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, Khan, and Saeed, (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· + scavenging capacity was compared with BHT and ascorbic acid. The percentage of inhibition was calculated as follows:

ABTS· + scavenging activity = (1 – (Abs sample) / (Abs control)) × 100

Where Abs sample is the absorbance of ABTS· + + sample (extract or standard) Abs control is the absorbance of ABTS· + + methanol.

**RESULTS:** The phytochemical contents of whole plant extracts of *Helichrysum petiolare* are shown in **Table 1**. According to the results obtained, the boiled aqueous extract (212.963 ± 0.260 mg/g) of *H. petiolare* had the highest total phenolic content compared to the other extracts.

The ethanol extract had very high phenolic contents, most importantly; it had the highest levels of flavonoids (172.39 ± 5.34 mg/g) and proanthocyanidins (65.86 ± 1.73 mg/g). The acetone (263.73 + 1.60 mg/g) extracts in this study also had the highest flavonol (143.87 + 0.55 mg/g), saponin (263.73 + 1.60 mg/g) and alkaloid (28 + 0.99 mg/g) contents compared to other extracts.

**TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF HELICHRYSUM PETIOLARE WHOLE-PLANT EXTRACTS**

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

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

The *in-vitro* antioxidant assay of the whole plant extracts of *H. Petiolare* showed significant and concentration-dependent total antioxidant capacities (TAC) compared with the standards ascorbic acid and BHT **Fig. 1**. The $IC_{50}$ of the extracts and standards against DPPH, ABTS, and nitric oxide radicals are shown in **Table 2**. According to this study, the four plant extracts had dose-dependent DPPH· + scavenging activities **Fig. 2**, their activities, however, were quite low compared to all of the standards at concentrations above 0.02 mg/mL, but they all had DPPH· + scavenging abilities that were greater than or equal to that of ascorbate at concentrations below 0.02 mg/mL.

**TABLE 2: SCAVENGING ACTIVITIES OF AQUEOUS (COLD AND BOILED), ACETONE AND ETHANOL WHOLE PLANT EXTRACTS OF *H. PETIOLARE***

| Activity | A | | B | | C | | | D |
|---|---|---|---|---|---|---|---|---|
| Samples | $IC_{50}$[a] | $R^{2}$[b] | $IC_{50}$[a] | $R^{2}$[b] | Samples | $IC_{50}$[a] | $R^{2}$[b] | $IC_{50}$[a] |
| Cold aqueous extract | 0.03 | 89.99 | 0.12 | 99.92 | Cold aqueous extract | 0.03 | 89.99 | 0.12 |
| Boiled aqueous extract | 0.02 | 72.47 | 0.07 | 99.8 | Boiled aqueous extract | 0.02 | 72.47 | 0.07 |
| Acetone extract | 0.02 | 67 | 0.19 | 99.94 | Acetone extract | 0.02 | 67 | 0.19 |
| Ethanol extract | 0.03 | 92.59 | 0.18 | 99.63 | Ethanol extract | 0.03 | 92.59 | 0.18 |
| Rutin | 0.02 | 94.76 | 0.0006 | 57.75 | Rutin | 0.02 | 94.76 | 0.0006 |
| Gallic acid | 0.55 | 78.04 | - | - | Gallic acid | 0.55 | 78.04 | - |
| Ascorbic acid | - | - | 0.03 | 79.5 | Ascorbic acid | - | - | 0.03 |
| BHT | 0.04 | 91.65 | 0.006 | 61.68 | BHT | 0.04 | 91.65 | 0.006 |

The letters represent; A= ABTS· + scavenging activity; B= DPPH· + scavenging activity; C=Nitric oxide scavenging activity; D=Total antioxidant capacity (TAC); a: $IC_{50}$ 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

The result also showed significant dose-dependent ABTS· + scavenging activities across all the extracts **Fig. 3**, the activities were much higher than those found in DPPH· + and highest for the 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 the plant extracts showed high nitric oxide radical inhibition greater than 80%. The activities of the extracts were highly competitive with those of the standards. The activities of the extracts were, however, non-dose dependent, except for the acetone and ethanol extracts, which showed a slight decline in activities with a rise in concentration.

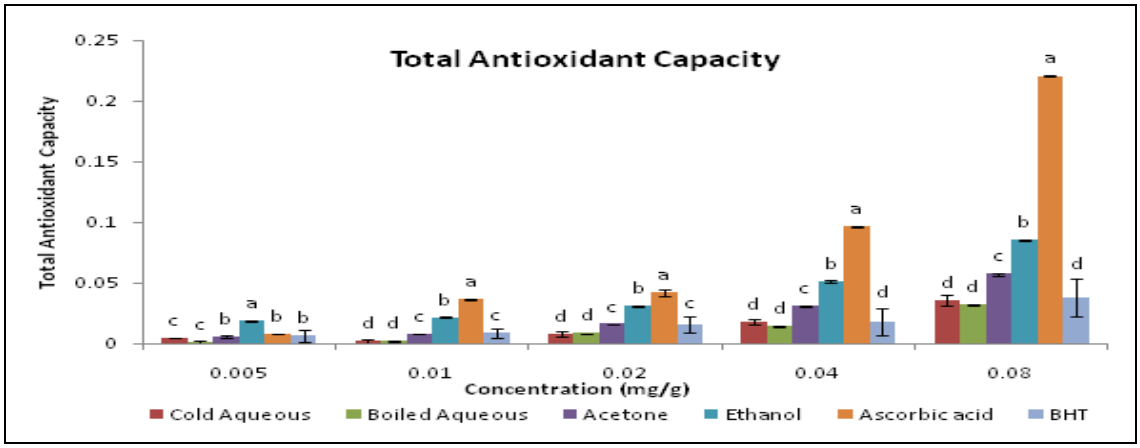


**FIG. 1: TOTAL ANTIOXIDANT CAPACITIES OF ASCORBIC ACID, BHT, COLD AND BOILED AQUEOUS, ACETONE AND ETHANOL WHOLE PLANT EXTRACTS OF *H. PETIOLARE*.** Data are presented as Means ± SD of three replicates. points with the same alphabet within the same concentration are not significantly different ($p < 0.05$)

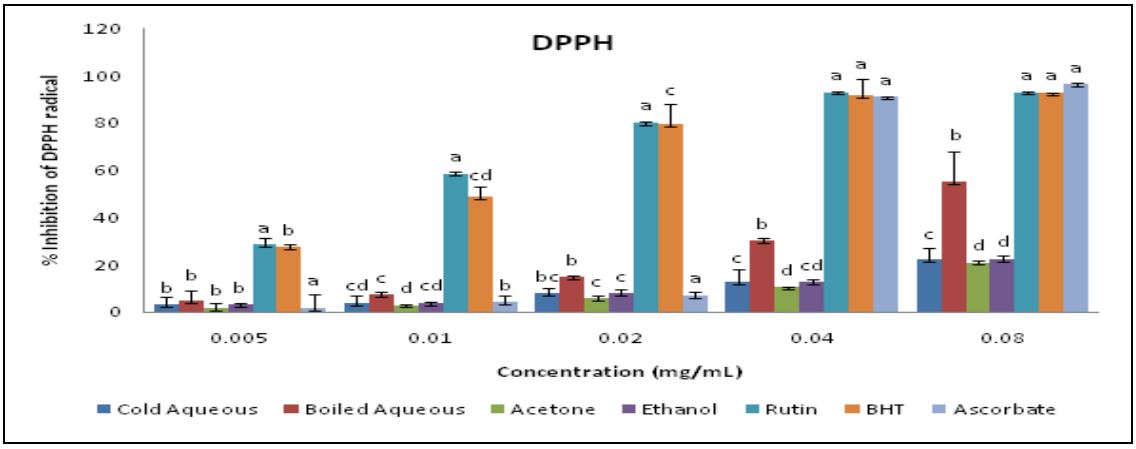


**FIG. 2: DPPH RADICAL SCAVENGING ACTIVITIES OF BHT, RUTIN, ASCORBATE, ETHANOL, ACETONE, BOILED AQUEOUS AND COLD AQUEOUS WHOLE PLANT EXTRACTS OF *H. PETIOLARE*.** Data are presented as Means ± SD of three replicates. bar graphs with the same letter superscripts within the same concentration are not significantly different ($p < 0.05$).

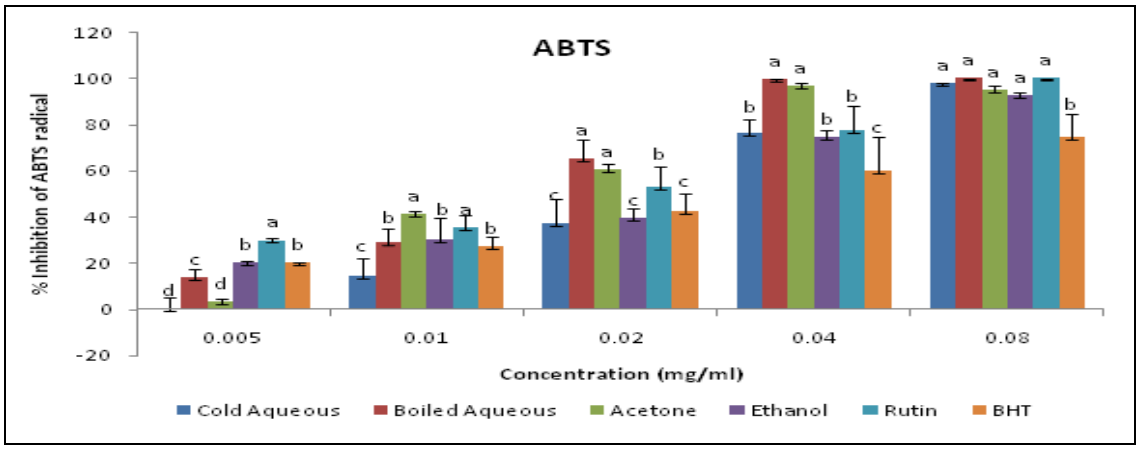


**FIG. 3: ABTS RADICAL SCAVENGING ACTIVITIES OF BHT, RUTIN, ASCORBATE, ETHANOL, ACETONE, BOILED AQUEOUS AND COLD AQUEOUS WHOLE PLANT EXTRACTS OF *H. PETIOLARE*.** Data are presented as Means ± SD of three replicates. bar graphs with the same letter superscripts within the same concentration are not significantly different ($p < 0.05$)

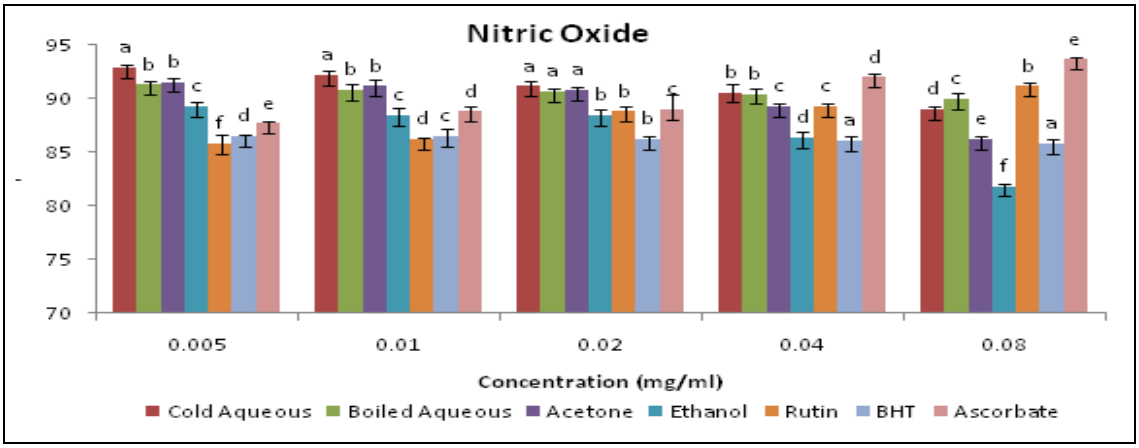


**FIG. 4: NITRIC OXIDE RADICAL SCAVENGING ACTIVITIES OF BHT, RUTIN, ASCORBATE, ETHANOL, ACETONE, BOILED AQUEOUS AND COLD AQUEOUS WHOLE PLANT EXTRACTS OF *H. PETIOLARE*.** Data are presented as Means ± SD of three replicates. bar graphs with the same letter superscripts within the same concentration are not significantly different ($p < 0.05$)

**DISCUSSION:** The analysis of acetone, ethanol, cold, and boiled aqueous extracts of the whole plant of *H. petiolare* showed the presence of saponin, alkaloids, flavonoids, flavonols, and proanthocyanidins. Proanthocyanidins are condensed tannins with various pharmacological properties [27]. Health-wise, they possess a wide range of beneficial properties, which include; antitumor, antibacterial, immune-stimulating, anti-allergic, antioxidant, antiviral, anti-carcinogenic, anti-inflammatory and vasodilatory properties [27]. Eating plants and fruits rich in proanthocyanidins have been shown by studies to help protect the body from sun damage, improve vision, flexibility in joints, arteries, and body tissues such as the heart, and to enhance blood circulation by strengthening the veins, capillaries, and arteries [28]. They can also inhibit platelet aggregation, lipid peroxidation, and capillary hyperpermeability [28, 29].

Flavonoids also have a wide range of biological activities, which include antioxidative, analgesic, anti-allergic, anti-angionic, antihypertensive, anticancer, antidiabetic, antimicrobial, and anti-inflammatory effects [30]. They also elicit their antioxidative properties by the inactivation of reactive oxygen species (ROS), thus counteracting plasma low-density lipoprotein (LDL) oxidation and consequently ameliorating inflammation of the blood vessel endothelium [31]. Recent studies have shown the health benefits of dietary flavonoids as there was a positive correlation between their intake and reduction of the risk of hypertension and cardiovascular death [32]. The very high flavonoids and proanthocyanidins contents of the ethanol extract must be due to the high extractive ability of the solvent and explain why the extract possesses the highest nitric oxide radical scavenging ability and total antioxidant capacity. This also supports the results obtained by Akinrinde, Afolayan, & Bradley, (2018) and further supports the usefulness of the ethanol extract of *H. petiolare* in the treatment and prevention of hypertension, treatment of cancer, fire injuries and other types of injuries, allergies, common infections, inflammation, insulin resistance, coronary heart disease, and diabetes. These results also explain why boiled aqueous extracts and ethanol herbal concoctions are preferable in the traditional herbal treatment, as high phenolic content has been shown by some authors to correlate strongly with high antioxidant activity [34,] and several reports have laid more importance on the key role of phenolic compounds as scavengers of free radicals [35].

Flavonols, flavones, and flavanols or catechins are three of the major subclasses of flavonoids [36], flavonols (*e.g.*, quercetin, kaempferol, and myricetin) are moderately absorbed in a gut with normal bacterial flora and are extensively metabolized in humans [37]. Flavonol is a flavonoid that has also been reported to have considerably high antioxidant properties. The previous study has associated increased activity of erythrocyte superoxide dismutase (antioxidant enzyme located in red blood cells) with the consumption of plants high in flavonol. Also, flavonol elicits an increase in plasma antioxidant capacity (the ability to scavenge free radicals), a decline in damage to lymphocyte DNA and a decrease in urinary 8-

hydroxy-2'-deoxyguanosine (a marker of oxidative damage) [38]. Flavonol has also been said by several studies to possess anticancer, anti-coronary heart disease, and anti-diabetic properties [39, 40]. This explains why the ethanol extract with very high flavonoid and flavonol content in this study had the highest total antioxidant activity **Fig. 1**. Therefore, *H. petiolare* especially, its acetone and ethanol extracts, may be effective in the prevention and treatment of cancer, coronary artery diseases, and diabetes.

The acetone and ethanol extracts in this study had the highest saponin contents, followed by the boiled aqueous extract. Saponins are heat-stable, amphiphilic, glycosidic compounds that are naturally present in a wide variety of plant food [41]. A previous study has shown that saponins enhance cardiovascular health due to their ability to reduce blood cholesterol and body fat levels; cholesterol absorption was also said to be inhibited by the consumption of plants rich in saponins by the saponins binding with the bile salts [42]. Saponins from ginger and ginseng have been shown in clinical trials, to reduce total and LDL (bad) cholesterol without altering HDL (good) cholesterol levels [42]. The acetone, ethanol and boiled aqueous extracts of H. petiolare, therefore, could be very useful in the prevention and treatment of hypercholesterolemia, hyperlipidemia, high blood pressure, atherosclerosis and cardiovascular diseases (CVD).

Despite reports from several authors that phenolic compounds are unstable and readily lose their antioxidant capacities once heated [43], the boiled aqueous extract exhibited the highest DPPH· + and ABTS· + scavenging activities **Fig. 2** & **3**, this may be due to the high extractive ability of the decoction method, and the presence of heat-stable viable antioxidant compounds. The total anti-oxidant capacity and nitric oxide radical scavenging ability of the boiled aqueous extract **Fig. 1** & **4** was, however, the lowest; this may have resulted from the loss of antioxidant power of the phenols due to heat instability. The strong free radical scavenging ability of all the plant extracts on ABTS· + in comparison to rutin and BHT suggests that there were some antioxidant compounds in the plant that can be isolated using water, ethanol and even acetone.

The acetone and boiled aqueous extracts exhibited the highest ABTS· + scavenging abilities **Fig. 3**; these high ABTS· + scavenging abilities were possibly due to the extracts' high saponin and flavonoid contents. The phenols of the boiled aqueous extracts were believed to have grossly reduced antioxidant properties, since a previous study has declared phenols as unstable losing their anti-oxidative abilities at high temperatures [43], saponins, however, are more heat-stable, showing no decline in the anti-oxidative property even at high temperatures [41]. The high ABTS radical scavenging abilities of the acetone and boiled aqueous extracts, therefore, imply that conditions caused by ABTS-like free radicals may be treated by traditional healers with the use of the plant's acetone and boiled aqueous extracts, and they can also use the extracts in the treatment of inflammations, cardiovascular diseases, arterio-sclerosis and hypercholesterolemia.

These observations were similar to the findings of Al-laith *et al.,* (2019), in which the compounds with high ABTS· + scavenging activities were reportedly displaying low DPPH· + scavenging activities. The result also showed that the four plant extracts used in this study had dose-dependent DPPH· + scavenging activities **Fig. 4**, their activities, however, were quite low compared to all of the standards at concentrations above 0.02 mg/mL, but they all had DPPH· + scavenging abilities that were greater than or equal to that of ascorbate at concentrations below 0.02 mg/mL, this means 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 nitric oxide inhibitory activities of the acetone, ethanol, boiled and cold aqueous extracts being higher than that of the BHT **Table 2** indicate the comparative ability of the extracts to reduce oxidative damage to some vital tissues in the body; this is in agreement with several other works that have been done on other Helichrysum species in which similar phytochemicals and antioxidant activities as found in this study were reported [45, 46]. Since NO also plays an important role in the pathogenesis of inflammation [47], *H. petiolare*, may, therefore, be quite effective in the treatment of hyperglycemia-induced inflammation and wound healing.

**CONCLUSION:** *H. petiolare* had high phenolic contents 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 those of BHT and well comparable to ascorbic acid and rutin. This study revealed some of the antioxidative and medicinal potentials of the plant. The plant may be used to address the problems of inflammation, atherosclerosis, insulin resistance, and cardiovascular diseases because it had strong scavenging abilities for ABTS, DPPH, and NO-like radicals, which shows that the plant extracts may act against oxidation processes in the human body. This could explain its usage as a herbal treatment plant in Eastern Cape to treat ailments such as asthma, coughs, pains, 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 phyto-chemical contents and antioxidant activities than the cold aqueous extract, which means ethanol and boiled aqueous herbal extracts, may be more potent/effective for herbal treatment than the cold aqueous extracts.

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