Determination of Phenolic and Antioxidant Activity of Helichrysum Melitense Extract

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Abstract: Plants produce secondary plant metabolites via biosynthetic pathways to defend themselves from pathogens, herbivores and environmental stressors. These secondary plant metabolites are very useful in the chemical industry as they are unique sources for various pharmaceutical products, such as food additives and medicinals, the latter due to antiviral, antibiotic, antioxidant, and antifungal properties that they show.

This investigation deals with *Helichrysum melitense*, an endangered endemic flowering species unique to the Maltese islands, and the effect of seasonal, defatting process and part of plant variation on its extract's phenolic content and antioxidant activity. Information regarding this plant is scarce, and this study will aim to give more insight regarding its phytochemicals and their properties, as well as helping profile Malta's biodiversity. Samples of this plant were taken from four different locations in Gozo (Ħekka, Qawra, Wardija and Ta’ Ċenc) during the winter and summer seasons. The samples were extracted from three different plant parts (leaves, root/stems and flowers) through one of two extraction methods. The extracts were then subjected to several assays for the quantification of phenolic content (TPC, TFC and ToPC) and antioxidant activity (DPPH, ABTS, CUPRAC, FRAP and OH).

Results showed that phenolics are higher in winter, while antioxidant activity showed mixed results. DPPH, ABTS and CUPRAC indicated that antioxidant activity is higher in winter, while FRAP and OH exhibited opposing results. Polar fractions showed higher phenolic content as well as antioxidant activity than defatted fractions. The highest phenolic content and antioxidant activity were found in leaves followed by root/stems and then flowers. Correlation analysis was performed and, overall, a positive relationship between phenolic content and antioxidant activity was established implying that antioxidant activity increases upon increased values of phenolic content, and decreased upon decreased values.

Keywords: Helichrysum, Maltese islands, endemic, antioxidants, free radicals, phenolic compounds

Introduction

Plants have long been studied and exploited for their various medicinal purposes. *Helichrysum melitense* (common name ‘Maltese Everlasting’ or ‘Sempreviva t’Ghawdex’) forms part of the Asteraceae (Sunflower) family and is an endangered endemic Maltese plant. This plant grows on rocks and has distinct silver-green foliage and an inflorescence of yellow composite flowerheads that appear in late spring to early summer (Pasta et al. 2017). During mid-summer, small dry seeds – achenes, are produced which bear a pappus to facilitate wind dispersal. These rupicolous shrubs usually range between 50-150 cm in diameter and 20-60cm in height. They also have lanceolate leaves enveloped by woolly hairs which protect it from sea spray and help it retain moisture during summer.
The main population of *H. melitense* is located on the west cliffs of the island of Gozo. A few isolated, smaller populations are also present inland west of Gozo and the south coast of Gozo and Malta. They abundantly colonize the higher parts of horizontal cliffs, but they also spread horizontally on top of the cliffs. *H. melitense* is closely related to the more widely spread curry plant (*Helichrysum italicum*) and this can be observed by the noticeable odour of curry when crushing the plant. Relatives of the Helichrysum species, such as *H. italicum* and *H. arenarium*, are widely used medicinally in the treatment of rheumatism and asthma, which may mean *H. melitense* exhibits similar active properties.

In recent years, this genus was the subject of several different pharmacological and phytochemical studies. Helichrysum species represent an abundant source of secondary...
metabolites. Secondary metabolites can be defined as organic compounds which aren’t directly involved in the natural growth, development and reproduction of the plant. In contrast to primary metabolites, absence of secondary metabolites does not result in the plants’ decease, but rather affects its aesthetics, fertility or chances of survival, or neither to a significant extent. Secondary metabolites show numerous biological effects, including antioxidant, antiviral, antibiotic and antifungal properties which help protect plants from pathogens.

Several different secondary plant metabolites can be found in *H. melitense*. The most common secondary plant metabolites are phenolics. Their structure is composed of at least one aromatic ring with one or more hydroxyl groups attached.

![Chemical structures of major phenols found in Helichrysum plants](image)

Plant phenolics provide structural integrity and play a key role in plant development, especially when it comes to biosynthesis of pigment and lignin. Plant phenolics help the plant defend itself from pathogens and ultraviolet radiation (Dai and Mumper 2010). An example of this is phenolic phytoalexins which are phenolic derivates that plants secrete once wounded. Their purpose is to aid the plant in repelling and killing any foreign detrimental microorganisms which might enter and cause further harm to the wounded area. Phenolics also contribute to the taste and colour of plants. Phenolics are highly valued pharmacologically for their antimicrobial and antioxidant properties (Bhattacharya et al. 2010).

This study was aimed to determine how such phenolic content and associated antioxidant activity in *H. melitense* extract is affected by seasonal, defatting process, and part of plant variation. It also aimed to verify whether a significant correlation between phenolic levels and antioxidant activity exists.

**Research Method**

Plant samples were taken from 4 different sub-locations found in the north-western region of Gozo (Ta’ Ċenċ, Wardija, Ħekka and Qawra) during the summer (August 2020) and winter (February 2021) seasons. The samples were left in a drying cabinet for 10 days at a temperature of 33°C. Once dried, the plants were manually separated into leaves, flower heads, and roots & stems. These were then blended separately into a fine powder.

Defatted fractions were prepared by mixing 3 grams of plant powder with 100 mL of hexane and leaving for 7 days in an automatic stirrer. The hexane fraction was vacuum filtered, and the filtrate was disposed of whilst the residue was kept and mixed with 100 mL methanol.
and left for a further 7 days in an automatic stirrer. The sample was then subjected to rotatory evaporation at 45°C, 120 rpm and 50 mBar. Once all the methanol evaporated, 10 mL of methanol was mixed in the round bottom flask containing the pure extract. To prepare polar fractions the same process was followed whilst omitting the hexane defatting stages. The following assays were used for both polar and defatted fractions on both summer and winter samples.

**Total Phenolic Content (TPC)**

A micro pipette was used to transfer 100 µL of Folin-Ciocalteu reagent (DF5) to a 96-well microtiter plate, to which 20 µL of the diluted extract were also added, followed up by adding 80 µL of Na₂CO₃ (7.5%). The microplate was then left for 2 hours in the dark and the absorbance was read at 630 nm by means of a microtiter plate reader. (Singleton et al., 1999). TPC was expressed as Gallic acid equivalents (mg/ml GAE). Three repetitions of each well were taken to obtain an average result.

**Total Flavonoid Content (TFC)**

The assay is based upon a method used by Mabry et al. (1970). 25 µL of the diluted extract (DF5) were placed in a well. The following quantities of chemicals were added to the diluted extract chronologically: 7.5 µL of 10% aluminium chloride, 7.5 µL of 7% w/v sodium nitrite, and 100 µL of 1M NaOH solution. After shaking the plate to ensure reaction had occurred, the absorbance of reaction was read using a spectrophotometer at 405 nm. A calibration curve was prepared against quercetin and the results expressed as mg quercetin equivalents (mg/ml QE).

**Total Ortho-Diphenolic Content (ToPC)**

Arnow’s reagent as described by Mateos et al. (2001), was prepared in situ by weighing 10 grams of sodium nitrite and 10 grams of sodium molybdate and mixing with 100 ml ethanol/water (1:1). The following quantities of chemicals were chronologically added to 20 µL of the diluted extract (DF5); 20 µL 1M hydrochloric acid, 20 µL Arnow’s reagent, 80 µL distilled water, 40 µL of 1M sodium hydroxide. The microplate was then read at an absorbance of 405 nm and the results recorded. A calibration curve was prepared against pyrocatechol and the results expressed as mg pyrocatechol equivalents (mg/ml PCE).

**DPPH Radical Scavenging Activity**

A solution of 60 µM DPPH was prepared in methanol and kept in a fridge at 4 °C. 25 µL of the stock extract solution was transferred to a well and two-fold serial dilution was carried out throughout the row of wells to further dilute the extract with methanol (25 µL). 250 µL of DPPH was then transferred to each well and subsequently the microplate was placed in the dark for an hour before taking the reading at 490 nm as suggested by Contreras-Guzman and Strong (1982). A column for negative DPPH control was prepared prior by adding 25 µL of stock extract solution into the wells and adding 250 µL of methanol.

The following equation was used to calculate the percentage of DPPH radicals scavenged by the extracts:

\[
\% \text{ Radical Scavenging} = \frac{Abs_{\text{DPPH in methanol}} - Abs_{\text{DPPH with sample}}}{Abs_{\text{DPPH in methanol}}} \times 100.
\]
ABTS Radical Cation Stabilization

The ABTS solution was prepared by mixing 2.45 mM potassium persulfate with 7 mM stock solution of 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and leaving the resulting solution in the dark at room temperature for approximately twelve hours. Methanol was used to adjust the concentration of the solution to the desired absorbance. 25 µL of the stock extract solution was transferred to a well and two-fold serial dilution was carried out throughout the row of wells to further dilute the extract with methanol (25 µL). 250 µL of ABTS was then transferred to each well and subsequently the microplate was placed in the dark for an hour before taking the reading at 405 nm (Cerretani and Bendini, 2010). A column for negative ABTS control was prepared prior by adding 25 µL of stock extract solution into the wells and adding 250 µL of methanol.

The percentage inhibition of ABTS was calculated as follows:

\[
\% \text{ ABTS}^+ \text{ inhibition} = 100 - 100 \left( \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right)
\]

CUPRAC Reducing Antioxidant Power Assay

A volume of 25 µL of the stock extract solution was transferred to a well and two-fold serial dilution was carried out throughout the row of wells as to further dilute the extract with methanol. This was carried out to determine the best possible dilution which gave optimal and coherent results. The following quantities of chemicals were then added to each well: 100 µL of 10 mM CuCl₂ solution, 100 µL of 1M ammonium acetate buffer (pH 7), 100 µL of 7.5 mM neocuproine methanolic solution. A column for negative CUPRAC control was prepared by adding 25 µL of stock extract solution into the wells and adding 300 µL of methanol. The absorbance was taken at 450 nm (Özyürek et al., 2011). CUPRAC was expressed as Gallic acid equivalents (mg/ml GAE).

Ferric Reducing Antioxidant Power (FRAP) Assay

The TPTZ-Fe³⁺ working solution was made by mixing 1 ml of 300.0 mmol/L acetate buffer with 1 ml of 10 mmol/L TPTZ solution and 1 ml of 20 mmol/L FeCl₃ solution, then diluting with methanol to 50 ml. This was done to prevent precipitation of extract in aqueous solution. For the assay, 50 µL of diluted extract (DF5) was put into a well and 200 µL of the TPTZ-Fe³⁺ working solution was added to it. Blanks (triplicates) were taken by mixing 200 µL of methanol with 50 µL diluted extract. The absorption was read with the microplate reader at 630 nm as suggested by Rajurkar and Hande (2011). A calibration curve was prepared against Gallic acid and the results expressed as mg Gallic acid equivalents (mg/ml GAE).

Hydroxyl Radicals Scavenging Activity

The method is based on Zhang et al. (2008), with slight modifications. The following quantities of chemicals were added to 25 µL of diluted extract chronologically: 10 µL of 1mM FeSO₄, 100 µL of methanol, 10 µL of 1M hydrogen peroxide and 15 µL of 1 mM phenanthroline. Addition of hydrogen peroxide initiates the reaction. Blanks (triplicates) were taken by mixing 135 µL of methanol with 25 µL diluted extract. Absorbance was read at 450nm. A calibration curve was prepared against Gallic acid and the results expressed as mg Gallic acid equivalents (mg/ml GAE).
Results and Discussion

Normality testing using Kolmogorov-Smirnov and Shapiro-Wilk tests was performed and the data were found to be non-normally distributed and hence non-parametric statistical methods were subsequently used. The Mann Whitney test was used to compare the median levels of phenolic content and antioxidant activity of two independent plant subpopulations.

Seasonal Variation

For seasonal variation, there was enough evidence to reject the null hypothesis, implying that a significant difference between summer and winter plant levels of secondary metabolites and antioxidant properties was observed. These results were consequent to various specific seasonal factors experienced in Malta’s ecosystem that affect secondary plant metabolite production, namely temperature, light irradiation, drought, and salinity.

In the Maltese islands, temperatures are much higher in summer (31°/22°, highest/lowest) when compared to winter (16°/9°, highest/lowest), (Galdies, 2011). In a study on soybean roots, phenolic and isoflavonoid levels were found to increase after treatment at low temperatures (Janas et al. 2002). Similarly, it is possible that the secondary metabolites in H. melitense, generated by the high temperature conditions during summer, might be enhanced by a continuously cooler temperature, thus producing more phenolics in winter. A possible explanation to why phenolics are higher in winter in H. melitense is that cold stress affects the phenylpropanoid/shikimate pathway responsible for the biogenetic synthesis of plant phenolics. This stimulation of phenolic biosynthesis occurs due to enhanced expression of CAD (cinnamylalcohol dehydrogenase), HCT (hydroxycinnamoyl transferase) and PAL (phenylalanine ammonia lyase) in cold conditions, and aids to prevent cell collapse and chilling injury (Sharma et al. 2019; Singh et al. 2021).

Another factor contributing towards differences in secondary metabolites is light irradiation in all its forms such as intensity, wavelength, duration, and frequency. All these factors are higher during summer in the Maltese islands. In these conditions phenolics are expected to increase since they accumulate in plant cells by forming a shield under the epidermal layer to protect plants from UV which generates harmful reactive oxygen species, damages the plants’ protein structures, and causes detrimental mutations to their DNA (Xu et al. 2017).
Since phenolics were higher in winter it can be concluded that photoperiod and light irradiation in all its forms must therefore not affect *H. melitense* as much as other environmental factors. A possible explanation to this is altitude. Umek et al. (1999) studied the relationship between altitude of plant and rutin concentration on *Hypericum perforatum* and reported that the amount of bioflavanoid found in plants at 200 metres above sea level was four times less than that found in plants at 800 meters above sea level. The study concluded that this is mainly due to the variance in solar radiation levels, as plants which are higher up are more exposed to UV than those at lower altitudes. The highest point in Gozo above sea level is only 190 metres, which means that light might not have been as intense as in other studies with sampling sites being potentially much higher in altitude. It can be speculated that this difference in ecology is the reason why light irradiation does not affect *H. melitense* as much as other abiotic factors.

The aforementioned factors can lead to drought stress which is a typical atmospheric condition experienced during the Maltese summer. Drought stress occurs when water availability is reduced to critical levels and atmospheric conditions promote further loss of water. This causes oxidative stress on plants which produce antioxidants to counter this. According to Azhar et al. (2011), water stress causes an increase in plant pigments and phenolics, with a decrease in biomass in *T. ammi*. In another study on *Hypericum Brasiliense*, water stress was found to improve key secondary metabolites such as betulinic acid, quercetin and rutin (Verma & Shukla, 2015). It can be concluded that drought is not an impactful external stress on *H. melitense* since although in summer it rains much less than in winter, phenolic levels in winter were still found to be higher. This was also observed by Antunes et al. (2018) who determined that *H. italicum* plants were not limited by water availability, even in summer. They state this is due to various adaptations where the plant focuses on conserving water by decreasing water loss such as stomatal control and high leaf pubescence which lowers light absorbance.

*H. melitense* is found on cliffs surrounded by large bodies of sea water. Taking this into consideration salt deposition is very likely to have contributed towards seasonal variation observed. Salt stress usually causes both osmotic and ionic stress in plants, leading to an accumulation or decrease of specific secondary metabolites in plants as a response to the stress. An experiment conducted on *Aegiceras corniculatum* which was treated by 250 mM NaCl, showed a double increase in polyphenol content as compared to control plants (Parida et al. 2004).

In relation to salinity, a further explanation as to why higher phenolic levels were found in winter in this study is that all the samples were taken from cliffs which are surrounded by the sea. Wells and Shunk (1938) emphasize salt spray as an important factor when investigating coastal ecosystems. The stronger winds in winter might lead to more salt being deposited onto the plants which are so close to the sea, thus helping to instigate higher phenolic levels. It is therefore possible that salt stress instigates *H. melitense*’s secondary metabolite production. Genes like Vv6HLH1 may be consequent to increased production of flavonoids in *H. melitense*, by regulating genes’ biosynthetic pathways and increasing salt tolerance in plants (Wang et al. 2016).
Fractional Variation

![Figure 5: A - Mean TPC (mg/ml GAE), B - Mean TFC (mg/ml QE) and C - Mean ToPC (mg/ml PCE) clustered by Fraction (polar = black, defatted = white) and part of plant.](image)

The defatting process used on *H. melitense* resulted in an overall decrease in the level of phenolic content, where polar fractions were found to generally contain more phenolics than defatted fractions. This coincides with the findings of Zishiri (2006) who conducted a study on various families of plants, one of them being *C. woodii*. From all the solvents used in the latter study, hexane extracted the least amount of plant powder and related antioxidant compounds, while ethanol extracted the highest amount. The DPPH assay was used, and it was found that hexane fractions exhibit no antioxidant activity at all.

This finding differs from observations corresponding to *H. melitense* since, in our case, defatted fractions were found to exhibit antioxidant activity, albeit to a lower extent than polar fractions. The results of fractional variation go hand in hand with the correlation analysis results, where phenolic and antioxidant activity were found to be positively correlated. Another reason for the results observed is that terpenes are composed of unsaturated hydrocarbons and are therefore non-polar, making them more soluble in hexane. This implies that the hexane fraction removes terpenes, potentially leading to defatted fractions exhibiting lower antioxidant activity. Further to this, terpenoids contain additional functional groups which usually contain oxygen, making them more polar. This indicates that they are more soluble in the polar fraction, further explaining why polar fractions exhibit higher antioxidant activity.

Part of Plant Variation

For part of plant variation, there is again enough evidence to reject the null hypothesis since a significant difference between phenolic levels and antioxidant activity between different parts of plants was observed. The order from highest to lowest levels of phenolics/antioxidant activity in *H. melitense* was found to be leaves followed by root/stems and then flowers.

Naghiloo et al. (2012) conducted research on roots, leaves and flowers of *Astralagus compactus Lam*, and concluded that the different plant parts exhibit different phenolic levels when applying the Folin-Ciocalteu assay. It was found that the highest phenolic content is in leaves followed by roots and flowers. This finding was in accordance with that of Niknam and Ebrahimzadeh (2002) who used similar spectrophotometric methods and found that phenolics are higher in leaves compared to roots and seeds.
The trend found in *H. melitense* is therefore the same as that found in the studies previously mentioned. It is thought that leaves produce phenolics to reduce photo-destruction of exposed tissues due to the intensity of solar radiation, and that this is the reason why they show higher phenolic levels compared to other plant parts (Mole & Waterman, 1988; Kotilainen et al. 2010). Another reason why leaves contain higher phenolic levels is that they are used by the plant as a defence mechanism to deter herbivores (Singh et al. 2021). In fact, flavonoids were found to reduce food consumptions of insects. An example of this was seen in soybean (*Glycine max* L.) leaves where quercetin 3- glucosylgalactoside and rutin reduced consumption by cabbage looper (Hoffmann-Campo et al. 2001). Another way in which the plants use leaves is antixenosis to deter herbivore through morphological adaptations such as colour, hairiness, wax on leaves, and by emitting foul-odour terpenes (Xing et al. 2017). Literature as well as this study’s correlation analysis showed that phenolics and antioxidant activity are positively correlated, so it can be concluded that antioxidant activity was lower in root/stems and more so in flowers due to the respectively decreasing phenolic levels in these plant parts.

**Antioxidant Activity & Phenolics Correlation Analysis**

Spearman rank-order correlation was used on the non-normal data to determine whether there exist significant associations between the levels of phenolics and antioxidant activity in each part of plant. Seasonal and fractional variations weren’t taken into consideration.

The p-value corresponding to each computed correlation coefficient was first examined. If this p-value exceeds the 0.05 level of significance, then no significant association is observed. Otherwise, the association between the phenolic and antioxidant assays under study is significant and can be interpreted with respect to strength and direction. All computed correlation coefficients and their corresponding p-values (in brackets) are displayed in Table 1. A reference table (Table 2) suggested by Mukaka (2012) follows, which was used to interpret the size of the correlation coefficients.

<table>
<thead>
<tr>
<th>Part of Plant/ Phenolic Assays</th>
<th>Antioxidant Assays</th>
<th>DPPH</th>
<th>ABTS</th>
<th>CUPRAC</th>
<th>FRAP</th>
<th>OH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaves</strong></td>
<td>TPC</td>
<td>-0.9415 (&lt;0.0001)</td>
<td>-0.5641 (&lt;0.0004)</td>
<td>0.8511 (&lt;0.0001)</td>
<td>0.1392 (0.4324)</td>
<td>-0.5308 (0.0004)</td>
</tr>
<tr>
<td></td>
<td>TFC</td>
<td>-0.9056 (&lt;0.0001)</td>
<td>-0.4894 (0.0052)</td>
<td>0.8453 (&lt;0.0001)</td>
<td>0.0990 (0.5962)</td>
<td>-0.5823 (0.0002)</td>
</tr>
<tr>
<td></td>
<td>ToPC</td>
<td>-0.8246 (&lt;0.0001)</td>
<td>-0.6594 (&lt;0.0001)</td>
<td>0.7188 (&lt;0.0001)</td>
<td>0.4039 (0.0197)</td>
<td>-0.2419 (0.1435)</td>
</tr>
<tr>
<td><strong>Root/Stems</strong></td>
<td>TPC</td>
<td>-0.7990 (&lt;0.0001)</td>
<td>-0.8058 (&lt;0.0001)</td>
<td>0.8569 (&lt;0.0001)</td>
<td>0.6628 (0.0011)</td>
<td>0.1957 (0.3279)</td>
</tr>
<tr>
<td></td>
<td>TFC</td>
<td>-0.8407 (&lt;0.0001)</td>
<td>-0.7601 (&lt;0.0001)</td>
<td>0.8445 (&lt;0.0001)</td>
<td>0.2265 (0.3511)</td>
<td>-0.1266 (0.5649)</td>
</tr>
<tr>
<td></td>
<td>ToPC</td>
<td>-0.6440 (&lt;0.0001)</td>
<td>-0.7944 (&lt;0.0001)</td>
<td>0.8490 (&lt;0.0001)</td>
<td>0.7439 (0.0003)</td>
<td>0.4108 (0.0413)</td>
</tr>
<tr>
<td><strong>Flowers</strong></td>
<td>TPC</td>
<td>-0.8005 (&lt;0.0001)</td>
<td>-0.7762 (&lt;0.0001)</td>
<td>0.9353 (&lt;0.0001)</td>
<td>0.6685 (0.0064)</td>
<td>-0.4697 (0.0044)</td>
</tr>
<tr>
<td></td>
<td>TFC</td>
<td>-0.8491 (&lt;0.0001)</td>
<td>-0.6506 (&lt;0.0001)</td>
<td>0.8772 (&lt;0.0001)</td>
<td>0.7286 (0.0021)</td>
<td>-0.7088 (&lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>ToPC</td>
<td>-0.6195 (&lt;0.0001)</td>
<td>-0.7887 (&lt;0.0001)</td>
<td>0.8723 (&lt;0.0001)</td>
<td>0.6321 (0.0115)</td>
<td>-0.1129 (0.5184)</td>
</tr>
</tbody>
</table>

*Table 1: Spearman rank-order correlation coefficients (and p-values in brackets) between phenolic and antioxidant assays*
### Size of Correlation Interpretation

<table>
<thead>
<tr>
<th>Size of Correlation</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9 to 1 (-0.9 to -1)</td>
<td>Very high positive (negative) correlation</td>
</tr>
<tr>
<td>0.7 to 0.9 (-0.7 to -0.9)</td>
<td>High positive (negative) correlation</td>
</tr>
<tr>
<td>0.5 to 0.7 (-0.5 to -0.7)</td>
<td>Moderate positive (negative) correlation</td>
</tr>
<tr>
<td>0.3 to 0.5 (-0.3 to -0.5)</td>
<td>Low positive (negative) correlation</td>
</tr>
<tr>
<td>0 to 0.3 (0 to -0.3)</td>
<td>Negligible correlation (insignificant)</td>
</tr>
</tbody>
</table>

**Table 2: Aid to interpret size of correlation coefficients**

For both DPPH and ABTS, the closer the correlation coefficient is to -1, the more antioxidant activity increases (or decreases) with an increase (or decrease) in the phenolic level. For the rest of the assays, the same principles apply the closer the correlation coefficient is to +1.

As can be observed in Table 4.1 for DPPH, ABTS, CUPRAC and FRAP a significant positive correlation was found in most cases meaning that the higher (or lower) the phenolic level, the higher (or lower) the antioxidant activity exhibited. For OH assay most of the significant correlations are negative, implying that, in contrast to the other four antioxidant assays, OH signifies that phenolic levels are inversely proportional to antioxidant activity. Overall, there is enough evidence to reject the null hypothesis, meaning that there is a significant correlation between phenolic levels and antioxidant activity.

### Conclusions and Scope for Further Research

It can be concluded that *H. melitense* and its secondary metabolites, like most of its relative species, contain phenolics and antioxidant properties. These properties were affected by seasonal, fractional, and part of plant variation. Both properties were found to be higher in winter and polar fractions. Leaves produced the highest amounts of phenolics followed by root/stems and flowers. Correlation analysis showed that a positive relationship exists between amount of phenolics and antioxidant activity. It is recommended that chromatographic analysis such as HPLC, GC and GC-MS be used in future research regarding *H. melitense* extract to identify phenolics and terpenoids respectively.

### References


