After the DPPH data was analyzed, it’s necessary to do a Gallic Acid (Polyphenol) Assay in order to identify the amount of polyphenols in our plant extracts. Gallic Acid is an antioxidant that also has a high polyphenol content. 20 standards were created using this known antioxidant, each having more polyphenols than the last (in mg/L). After their extractions and evaporation of the solvent, 25 grams of Melissa officinalis were used for each extraction condition.

**RESULTS**

<table>
<thead>
<tr>
<th>Table 1. Percent Yield</th>
<th>1 day at room temperature (RT)</th>
<th>7 days at room temperature (RT)</th>
<th>4 hours at 40°C then stirring for 24 hours at room temperature (RT)</th>
<th>2 days (48 hours) at 90°C</th>
<th>10 minutes in a microwave oven</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.9%</td>
<td>37.8%</td>
<td>45.6%</td>
<td>9.5%</td>
<td>39.4%</td>
</tr>
</tbody>
</table>

**DISCUSSION**

From our results, it can be said that the 1 day RT and 60:40 ethanol/water solvent was the best extraction condition. It is hypothesized that this solvent was able to extract both hydrophilic and hydrophobic compounds from our plant, adding to the extract’s ability to quench free radicals. When ethanol was used as a solvent, the 90°C for 4 hours condition showed the most antioxidant activity. This might have taken place as ethanol is a volatile solvent, so the extreme heat was able to extract more of the plant compounds. This can also be inferred as the highest phenolic content was recorded in the 40°C 4 hrs/RT 24 hrs condition with ethanol as the solvent.

**FUTURE WORK**

Future work on Melissa officinalis will lead to studies of the active compounds within this plant and test its efficacy with in vivo studies. This will be done with the purpose of discovering a potential treatment for Neuroblastoma with the use of Melissa officinalis. We hope this research sparks more studies for drug discoveries with the use of everyday plants.

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We would like to acknowledge our Scientist, Dr. Skouta for mentoring us in this journey and our RA Mary E. Fuentes for working with us and guiding us to complete this experiment.

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**NEUROBLASTOMA:** New Insights for the Healthcare Professional: 2011 Edition (Google Books)

Neuroblastoma (- Mayo Clinic)

Evaluating the Antioxidant Activity of *Melissa officinalis* via colorimetric assays to assess *in vitro* studies of Neuroblastoma

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**ABSTRACT**

*Melissa officinalis* (Lemon balm) is a mint plant that originated from Southern Europe. The purpose of our experiment was to find which extraction condition would yield the greatest antioxidant activity from *Melissa officinalis* using different extraction conditions and solvents. In a past study about colon cancer, ethanolic extracts of *Melissa officinalis* revealed antiproliferative and antioxidant properties. With this information, it was hypothesized that the highest antioxidant activity of *Melissa officinalis* would be seen in the extraction condition of 40°C, for 4 hours/room temperature for 24 hours with ethanol as the solvent. The antioxidant properties of *Melissa officinalis* were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable free radical and Gallic Acid (Polyphenol) assays. After testing, it was found that our hypothesis was incorrect. The highest antioxidant activity was seen from the 1 day extraction with the 60:40 ratio of ethanol: water as the solvent. However, the highest polyphenol content was seen from the microwave condition using an ethanol solvent. With this data, the water extract from the 40°C condition was used to assess its efficacy against SH-SY5Y, a cell line derived from the bone marrow of a 4 year old female with neuroblastoma.

**METHODS**

Before assessing antioxidant activity, *Melissa officinalis* was extracted in these different conditions:

- 1 day at room temperature (RT)
- 7 days at room temperature (RT)
- 4 hours at 40°C then stirring for 24 hours at room temperature (RT)
- 2 days (48 hours) at 90°C
- 10 minutes in a microwave oven

After these extractions were done, the extracts were filtered using Celite. A DPPH assay was performed to measure the antioxidant activity of the plant extracts:

- The purpose of the DPPH assay was to measure our plant extract's quenching capabilities as it reacted with the DPPH free radical. A color change from dark violet to yellow can be seen when the free radicals are quenched by an antioxidant. The absorbance of these capabilities were seen with a spectrometer at 517 nanometers.
- A Gallic Acid (Polyphenol) Assay was also performed in order to quantify the amount of polyphenols in our plant extracts:
  - Gallic Acid is an antioxidant that also has a high polyphenol content. Standards were created using this known antioxidant, each having more polyphenols than the last (in mg/L). All five conditions were read on the assay so that they can be compared with the standard in order to record the amount of polyphenols in each extract.
  - This assay was read at 735 nm with the use of a spectrometer.

After the Polyphenol Assay, the plant extract was tested in the cell line to assess cytotoxicity and neuroprotection.

**RESULTS**

- Figure 1: *Antioxidant Activity from 40°C 4 hrs/ RT 24 hrs*.
  - The highest antioxidant activity for the 40°C 4 hrs/ RT 24 hrs was seen in TGS-7-52 (95.8%), which used water as the solvent.
  - This proved our hypothesis to be incorrect; since it was predicted that the ethanol solvent under this condition would reveal the highest antioxidant activity.

- Figure 2: *Antioxidant Activity from 60°C 48 hrs*.
  - The highest antioxidant activity in 4 hrs/ extraction condition was seen in TGS-1-5-52 (94.6%), which used ethanol as the solvent.

- Figure 3: *Antioxidant Activity from RT 1 day*.
  - The highest antioxidant activity in 1 day extraction condition was seen in TGS-1-5-53 (93.9%), which used a ratio of 60:40 ethanol water as the solvent.

**DISCUSSION**

- Table 1. Percent Yield
  - 25 grams of *Melissa officinalis* leaves were used for each extraction condition. After their extractions and evaporation of the solvent, a percent yield was calculated to find how much of the plant was extracted.

- Table 2. Folin-Ciocalteau Gallic Acid Equivalent Values
  - This table shows the polyphenol content seen in Figure 5.

- Figure 4. *Assessing Cytotoxicity using SH-SY5Y*.
  - This was done with the purpose of discovering a potential treatment for Neuroblastoma through *in vitro* studies using the SH-SY5Y cell line.

**FUTURE WORK**

Future work on *Melissa officinalis* will lead to studies of the active compounds within this plant and test its efficacy with in vivo studies. This will be done with the purpose of discovering a potential treatment for Neuroblastoma with the use of Melissa officinalis. We hope this research sparks more studies for drug discoveries with the use of everyday plants.

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**REFERENCES**


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