


# Antioxidant and Antibacterial Activities of the Extracts from the Leaves of Sweet Fern (*Comptonia peregrina* L. Coult)

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A decorative teal silhouette of a mountain range is located in the bottom right corner of the slide.

# Why This Study?

- ◆ **Plants : an important component of peoples' medicine, also used as food, sweeteners, preservatives, flavorings and beverages**
  - ◆ **To study uninvestigated (or less investigated) medicinal plants and herbs used by the First Nations of Northern Ontario in the search for potential pharmacological natural compounds, more effective and non-toxic.**
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# Why Antioxidants?

- ◆ **Oxidation in our body : caused by free radicals produced as normal byproducts of cell metabolism.**
  - ◆ **These free radicals play an important role in cardiovascular diseases, aging, cancer, and a variety of other disorders.**
  - ◆ **Food containing high percentages of antioxidant compounds can reduce the risk of these diseases.**
  - ◆ **There is a growing interest in antioxidants extracted from natural sources, mostly of edible plant origin.**
  - ◆ **Diet plays a definite role in the onset of diabetes, cardiovascular diseases and even cancer.**
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# The Plant: *Comptonia peregrina*

## 1. Description :

- Genus : *Comptonia*
- Family : Myricaceae, order Fagales.
- Distribution: grows wild in eastern North America
- Common name is Sweet-fern.

## 2. Economic importance:

- Edible Uses : Condiment; Fruit; Tea.



# The Plant: *Comptonia peregrina* (continued)

## - Medicinal Uses

❖ Astringent

❖ Blood purifier

❖ Expectorant

❖ Febrifuge

❖ Odontalgic

❖ Parasiticide

❖ Poultice

❖ Tonic

## – Other Uses

◆ Incense; Repellent.

# Material and Methods

## ◆ Plant material :

- Air- dried and powdered leaves

## ◆ Methods:

- Soxhlet Extraction

- Successive solvents fractionation

- Antioxidants assays

ORAC (Oxygen Radical Absorbance Capacity)

DPPH (2,2-DiPhenyl-1-PicrylHydrazyl )

- Antibacterial assays

Disk diffusion technique




# Extraction


## ◆ Soxhlet :

- ◆ Plant material : leaves (401g)
- ◆ Solvent: Ethanol (85%)
- ◆ Extraction : 24 hours
- ◆ Crude extract : 122g

## ◆ Fractionation:

- ◆ Hexane : 28.3389g
  - ◆ Dichloromethane: 4.9691g
  - ◆ Ethyl Acetate: 10.8559g
  - ◆ Butanol: 15.3307g
  - ◆ Ethanol : 37.3165g
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# Essential Oil

- ◆ **Plant material : dried powdered leaves (549g)**
  - ◆ **Extraction: steam distillation( 4 hours)**
  - ◆ **Yield: 0.1g (0.02%)**
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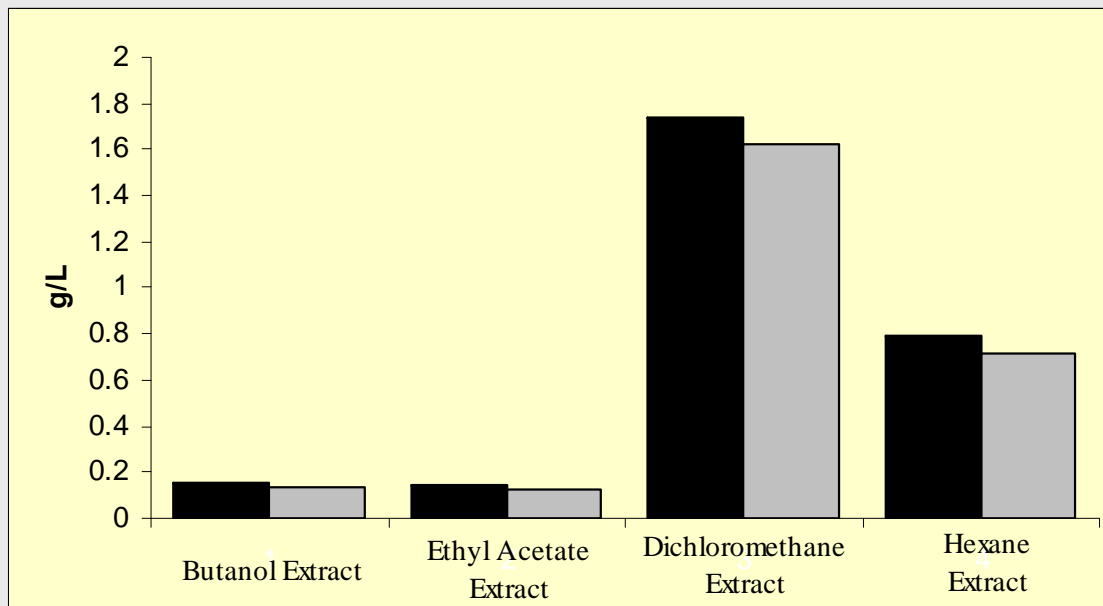
# Antioxidant Assays

## 1. DPPH

- **Sample solutions: 6mL 65 $\mu$ M of DPPH dissolved in methanol and 154 $\mu$ L of methanolic solution extracts (0.1, 0.15, 0.2, 0.25 g/L).**
- **Methanol was used to zero the instrument.**
- **Absorbance readings: 30 minutes and 60 minutes.**
- **Standard curves : generated for each extract by plotting concentration versus inhibition**
- **Results : interpreted as EC<sub>50</sub> of each extract from calculations using the formula generated from the linear line of best fit.**

# Results

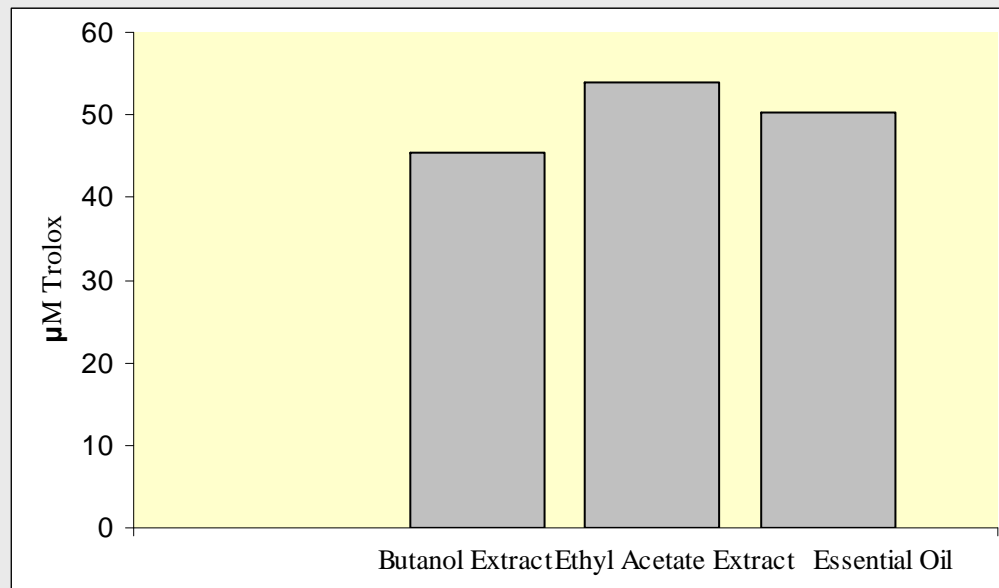
- ◆ The  $EC_{50}$  of the extracts ranged from:
  - 0.1465 to 1.7379 g/L of extract after 30min incubation time (black)
  - 0.12198 to 1.6251 g/L of extract after 60min incubation time (grey).
  - Dichloromethane extract exhibits the lowest antioxidant activity
  - Ethyl acetate extract shows the highest



# Antioxidant Assays (continued)

## 2. ORAC:

- The antioxidant capacity of the extracts ranges from  $45.313 \pm 0.104\%$  to  $53.951 \pm 0.856\%$   $\mu\text{M}$  TROLOX equivalents per 0.01 g/L of extract
- Ethyl acetate extract exhibits the highest.



# Antibacterial Assays

## ◆ Samples tested:

- Butanol, ethyl acetate, dichloromethane extracts
- Essential oil

## ◆ Bacterial strains used :

- *Bacillus subtilis*
- *Streptococcus mutans*
- *Alcaligenes faecalis*
- *Pseudomonas fluorescens*
- *Escherichia coli* (ATCC 25922),
- *Salmonella enterica* (ATCC 13311)
- *Bacillus cereus* (ATCC 11778)
- *Staphylococcus aureus* (ATCC 25923)

## ◆ Method used: disk diffusion technique

# Antibacterial Assays : Results

	Butanol	Ethyl Acetate	Dichloromethane	Essential Oil
<b>Gram Positive</b>				
<i>Bacillus cereus</i>	123.8 ±4.8	128.6 ±0.0	114.3 ±8.2	119.0 ±12.6
<i>Bacillus subtilis</i>	138.9 ±23.4	133.3 ±20.8	142.9 ±21.8	184.5 ±50.9
<i>Staphylococcus aureus</i>	142.9 ±14.3	164.3 ±7.1	147.6 ±12.6	100.0 ±0.0
<i>Streptococcus mutans</i>	122.6 ±11.4	136.7 ±13.1	111.1 ±23.1	143.9 ±20.6
<b>Gram Negative</b>				
<i>Alcaligenes faecalis</i>	149.8 ±13.3	239.1 ±45.5	105.0 ±10.4	216.8 ±48.1
<i>Escherichia coli</i>	118.0 ±5.4	136.0 ±6.9	118.0 ±5.4	116.9 ±8.7
<i>Pseudomonas fluorescens</i>	125.0 ±12.5	100.0 ±0.0	106.3 ±6.3	143.8 ±6.2
<i>Salmonella enteritidis</i>	119.0 ±9.5	123.8 ±12.6	119.0 ±9.5	114.3 ±8.2

# Antibacterial Assays : Discussion

- ◆ The antibacterial assays showed that:
  - Butanol extract exhibited an effect on all bacteria tested :
    - ◆ the greatest impact was on *Staphylococcus aureus* and *Alcaligenes faecalis*.
  - Ethyl acetate:
    - ◆ had no affect on *Pseudomonas fluorescens*
    - ◆ affect all other strains tested
    - ◆ *Staphylococcus aureus* and *Alcaligenes faecalis* showed the greatest inhibition of growth out of all the tested strains.

# Antibacterial Assays : Discussion (continued)

## – Dichloromethane extract showed:

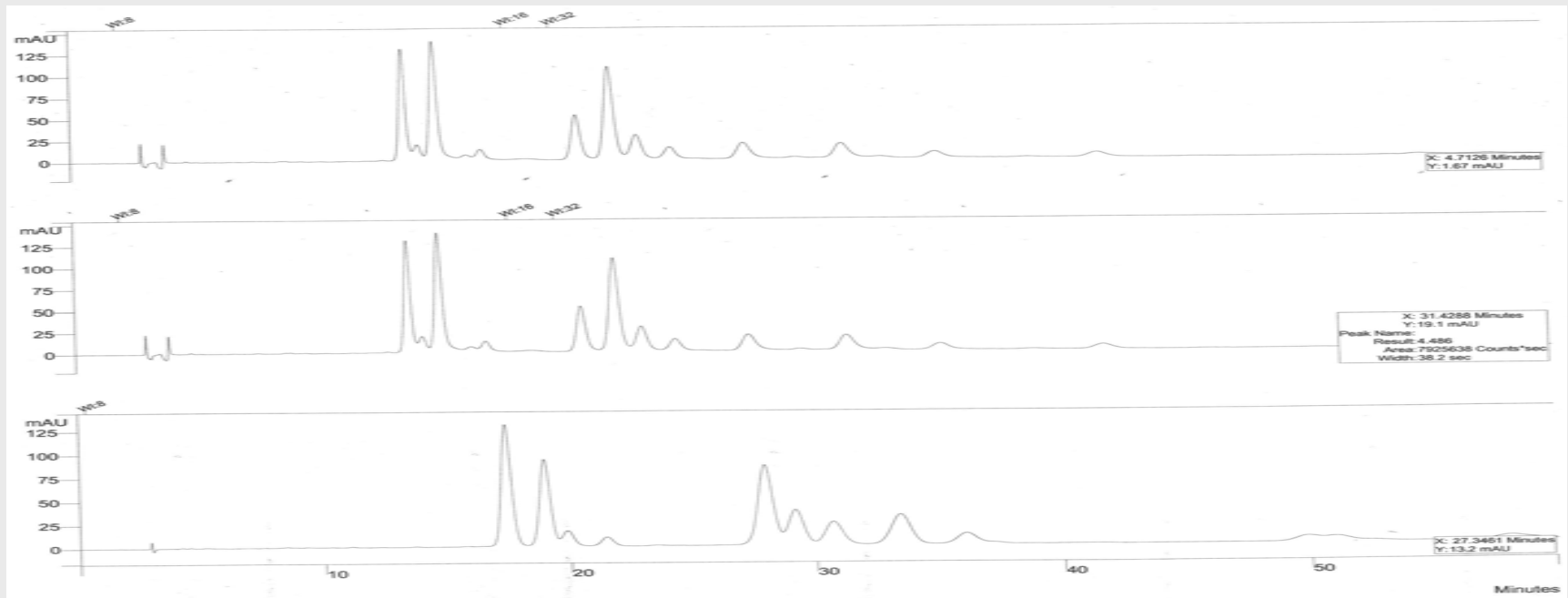
- ◆ No significant inhibition of *Alcaligenes faecalis*, *Pseudomonas fluorescens*, or *Streptococcus mutans*
- ◆ The largest effect on *Staphylococcus aureus* and *Bacillus subtilis*.

## – Essential oil extract:

- ◆ All strains showed inhibition of growth except for *Staphylococcus aureus*
  - ◆ *Bacillus subtilis* and *Alcaligenes faecalis* were the most inhibited.
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# HPLC Analysis of the EtOAc Extract

- ◆ Eluent : MeOH/H<sub>2</sub>O/TFA
- ◆ Chromatogram:



# Conclusion

## ◆ Antioxidant activity:

- Extracts from *Comptonia peregrina* leaves exhibit a strong antioxidant activity; Ethyl acetate extract has the highest activity, followed by butanol extract.

## ◆ Antibacterial activity:

- *Bacillus subtilis*, *Staphylococcus aureus*, and *Alcaligenes faecalis* are affected the most by extracts from sweet fern.
  - Butanol, ethyl acetate, and dichloromethane extracts strongly inhibit the growth of *Staphylococcus aureus*, a bacterial strain which causes skin infections.
  - This may explain the uses of the sweet fern by the First Nations peoples in the treatment of skin lesions and other infections .
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