Estimation of chlorophyll content in common household medicinal leaves and their utilization to avail health benefits of chlorophyll.

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ABSTRACT
Recent research shows that chlorophyll has many health benefits due to its structural similarity to human blood and its good chelating ability. It has anti-mutagenic and anti-carcinogenic properties. Tulsi (Ocimum tenuiflorum L), neem (Azadirachta indica L), curry leaves (Murraya koenigii L) and mint (Mentha arvensis L) are some common medicinal plants which are easily available in our households. The chlorophyll content in each was estimated by spectrophotometric method by using 80% acetone as a solvents as described by S. Sadasivan and A. Manickam (1991)."Biochemical methods". The aim was to determine which of these plants could provide a natural source of chlorophyll so as to attain the health benefits of chlorophyll readily without using any chemical supplements. In this work, health benefits of chlorophyll were studied on the leaves of four plants, Tulsi, neem, mint and curry leaves.

Key words: Tulsi, neem, mint, curry leaves, chlorophyll, acetone, centrifuge, absorbance.

INTRODUCTION
Since time immemorial mankind has utilized plants for their medicinal values. Several branches of science like ayurvedic medicine, unani medicine etc put paramount amount of importance on plant extracts for the cure of multitude of diseases. Tulsi (Ocimum tenuiflorum L), neem (Azadirachta indica L), curry leaves (Murraya koenigii L) and mint (Mentha arvensis L) are some common medicinal plants which are easily available in our households. Tulsi is used in Thai cuisine[1] and it has several medicinal benefits like reduction in total cholesterol levels and reduction of blood sugar levels thus controlling diabetes[2]. Tulsi's extracts are used in ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning, and malaria as mentioned by Charaka in Charaka Samhita[3]. Neem is called the "Sacred Tree" in India as almost all parts of the tree have medicinal benefits. Neem leaf paste is used to treat acne. It has antifungal, antibacterial and antiviral properties[4]. Mint has several medicinal uses in treating skin problems. It is especially useful in treating acne, itching, inflammation, and burns. Internally, it is considered excellent for indigestion and gastrointestinal problems[5]. Curry leaves helps in treatment of diarrhea, indigestion and nausea. It also helps in weight loss and prevents development of cataract and premature graying of hair.

Recent research now shows that chlorophyll has medicinal properties. Estimating the chlorophyll content in these plants could portray how chlorophyll plays an important part in their medicinal properties. Here are some of the known chlorophyll benefits[6]. It has been seen to help in the growth and repair of tissues. Chlorophyll helps in neutralizing the pollution that we breathe in and intake every day - a good supplement for smokers. It efficiently delivers magnesium and helps the blood in carrying the much needed oxygen to all cells and tissues. It also found to be useful in assimilating and chelating calcium and other heavy minerals. It had been seen to have a good potential in stimulating red blood cells to improve oxygen supply. Along with other vitamins such as A, C, and E, chlorophyll has been seen to help neutralize free radicals that do damage to healthy cells. Chlorophyll is also an effective deodorizer to reduce bad breath, urine, fecal waste, and body odor. It may reduce the ability of carcinogens to bind with the DNA in different major organs in the body. Chlorophyll may be useful in treating calcium oxalate stone ailments. It possesses some anti-atherogenic activity as well. It can be used to treat infected wounds naturally. It has anti-mutagenic and anti-carcinogenic properties so that it may be helpful in protecting your body against toxins and in reducing drug side effects.

Estimating the chlorophyll content in common medicinal leaves could help understand their medicinal properties better. They can be an easily available and cheap source of chlorophyll along with their additional medicinal value. They can be utilized naturally without any side effects. In case of mint and curry leaves they can be incorporated into our diet. The medicinal plants chosen for this study are readily available and inexpensive.

MATERIALS AND METHODS

Plant material
Healthy fresh green leaves of tulsi (Ocimum tenuiflorum L), neem (Azadirachta indica L), curry leave (Murraya koenigii L) and mint (Mentha arvensis L) were collected and 1 g of each was weighed and finely cut.

Extraction of chlorophyll
Chlorophyll was estimated as described by S. Sadasivan and A. Manickam (1991)."Biochemical methods". 1 g of finely cut fresh leaves were ground to a fine pulp with the addition of 20 ml of 80% acetone with a mortar and pestle. This paste was the centrifuged for 5 minutes at 5000 rpm. The supernatant was transferred to a 50 ml beaker. The residue was then ground with 20 ml of 80% acetone, centrifuged for 5 minutes at 5000 rpm and the supernatant was transferred to the same beaker. This process was repeated for 4 times till the residues became almost colorless. The inside of the mortar and pestle were also washed with 80% acetone and the clear washings were also collected in the beaker. The volume was made up to 100 ml with 80% acetone. This was repeated for all the leaf samples. The absorbance of the extract solutions were read at 645, 663 and 652 nm against the solvent (80% acetone) blank.

Calculations
The amount of chlorophyll present in the extract i.e. mg of chlorophyll present per gram of tissue was calculated using the following equations as described in S. Sadasivan and A. Manickam (1991). "Biochemical methods":

\[
\text{Milligrams of Chlorophyll a per gram of tissue} = \left[12.7(A_{645}) - 2.69(A_{663})\right] \times \left(\frac{1000}{V}\right)
\]

\[
\text{Milligrams of Chlorophyll b per gram of tissue} = \left[22.9(A_{645}) - 4.68(A_{663})\right] \times \left(\frac{1000}{V}\right)
\]

\[
\text{Total chlorophyll per gram of tissue} = \left[20.2(A_{645}) + 8.02(A_{663})\right] \times \left(\frac{1000}{V}\right)
\]

Here \(A\) = absorbance at specific wavelengths, \(V\) = final volume of chlorophyll extract in 80% acetone which in the case is 100 ml and \(W\) = fresh weight of tissue extracted which is 1 g. Thus \(V/W = (1000/W) = 100\left(1000/W\right) = 0.1\).

Therefore the amounts of chlorophyll present in the respective sample are calculated as follows:

**Tulsi**
Chlorophyll a (mg) = \([12.7(1.458) - 2.69(0.775)] \times 0.1 = 1.6486\) mg
Chlorophyll b (mg) = \([22.9(0.775) - 4.68(1.458)] \times 0.1 = 1.0466\) mg
Total chlorophyll (mg) = \([20.2(0.775) - 8.02(1.458)] \times 0.1 = 2.7348\) mg

**Neem**
Chlorophyll a (mg) = \([12.7(5.440) - 2.69(3.320)] \times 0.1 = 6.0157\) mg
Chlorophyll b (mg) = \([22.9(3.320) - 4.68(5.440)] \times 0.1 = 5.0268\) mg
Total chlorophyll (mg) = \([20.2(3.320) - 8.02(5.440)] \times 0.1 = 11.0692\) mg

**Mint**
Chlorophyll a (mg) = \([12.7(3.573) - 2.69(1.638)] \times 0.1 = 4.0965\) mg
Chlorophyll b (mg) = \([22.9(1.638) - 4.68(3.573)] \times 0.1 = 2.0788\) mg
Total chlorophyll (mg) = \([20.2(1.638) - 8.02(3.573)] \times 0.1 = 6.1743\) mg

**Curry leaves**
Chlorophyll a (mg) = \([12.7(8.482) - 2.69(2.587)] \times 0.1 = 5.4354\) mg
Chlorophyll b (mg) = \([22.9(2.587) - 4.68(8.482)] \times 0.1 = 3.6581\) mg
Total chlorophyll (mg) = \([20.2(2.587) - 8.02(8.482)] \times 0.1 = 9.1090\) mg

RESULTS AND DISCUSSION

**RESULTS**
The absorbance readings of the extract of the samples against 80% acetone blank are presented in the table.
Table 1: Chlorophyll content of samples

<table>
<thead>
<tr>
<th>S No</th>
<th>Sample</th>
<th>Chlorophyll a (mg)</th>
<th>Chlorophyll b (mg)</th>
<th>Total chlorophyll (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tulsi</td>
<td>1.6486</td>
<td>1.0466</td>
<td>2.7348</td>
</tr>
<tr>
<td>2</td>
<td>Neem</td>
<td>6.0157</td>
<td>5.0268</td>
<td>11.0692</td>
</tr>
<tr>
<td>3</td>
<td>Mint</td>
<td>4.0965</td>
<td>2.0788</td>
<td>6.1743</td>
</tr>
<tr>
<td>4</td>
<td>Curry leaves</td>
<td>5.4534</td>
<td>3.6581</td>
<td>9.1090</td>
</tr>
</tbody>
</table>

DISCUSSION
It was found that neem leaves have the highest chlorophyll content followed by curry leaves. Thus they can be used as a relatively cheap and very easily available source of chlorophyll instead of chlorophyll supplements for availing the health benefits of chlorophyll compound. Curry leaves are an integral part of the Indian cuisine. Thus incorporating into our diets will aid in growth and repair of tissues and also possess anti mutagenic and anti carcinogenic properties to a certain extent as it is a very good chelating agent due to its physical and chemical structure. Thus we don’t have to rely on external chemicals or supplements for these benefits in keeping our body healthy and for increasing our immunity. Also they stimulate red blood cells in uptake of oxygen and efficiently deliver magnesium ions to our body. All these health benefits can easily be availed while utilizing the medicinal values also.

CONCLUSION
After estimating the chlorophyll content of Tulsi (*Ocimum tenuiflorum* L.), neem (*Azadirachta indica* L.), curry leaves(*Murraya koenigii* L.) and mint(*Mentha arvensis* L.) by using spectrophotometric methods using acetone as the solvent, it was found out that neem (*Azadirachta indica* L) has the highest chlorophyll content among the four followed by curry leaves(*Murraya koenigii* L) and thus these leaves can be utilized to avail the health benefits of chlorophyll compound along with their other medicinal benefits. They are also easily available and inexpensive.

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