Estimation of Chlorophyll From Aeromatic Medicinal Plants Used As Potent Therapeuritic Agents In Anaemic Patients.

1 Heema Patel, 2 Dr. Inampudi Sailaja, 3 Dr. Ivvala Anand Shaker
1 PG student, 2 Assistant Professor, 3 Professor and HOD
1 Department of Microbiology, 2 Department of Biochemistry

Parul Institute of Applied Science, Parul University, Limda, Waghodia dist. Vadodara, Gujarat, India, 3 PIMSR, Parul University, Limda, Waghodia-391760 dist. Vadodara, Gujarat, India.

Abstract: Chlorophyll is a green pigment, which is structurally similar to porphyrin pigments such as heme and it is produced through the same metabolic pathway in plants. Chlorophyll benefits the body in a unique and distinctive ways. It is helps to cleans harmful toxins from the body and it is also used to fight infection. Chlorophyll antiviral effects make it a strong skin protector. A recommended and regular intake of chlorophyll can keep the circulatory and digestive system much healthier. The present study, the chlorophyll was extracted from the different medicinal and aromatic plants, and characterized by UV-visible spectroscopy. These plant juice extract contains rich chlorophyll (whose structure is similar to hemoglobin except the central moiety), Amino acids, Minerals (like iron), Vitamins (like B12) and active enzymes are present. These juices' were used as therapeutic agents for treatment of various problems including Anemia, Thalassemia, etc. In our study we treated anemia with intake of three different juices for three different groups and found a significant results in treating anemia.

Keywords - Chlorophyll, Medicinal plant, Aromatic plant, Therapeutic use, UV-visible spectroscopy.

I. INTRODUCTION

A tetrapyrrole ring with a central magnesium ion and long hydrophobic phytol chain in structure is called a green pigment (chlorophyll) It is found in some varieties in plants and algae [1] since time immemorial mankind has utilized plants for their medicinal values.

Several branches of science like ayurvedic medicine, unani medicine etc put supreme amount of significant plant extracts for the heal of mass of diseases. Coriander (Coriandrum sativum), curry leaves (Murraya koenigii L) and mint (Mentha arvensis L) are some common medicinal plants which are easily available in our households.

Figure 1 (Coriander (Coriandrum sativum), Curry leaves (Murraya koenigii L) and Mint (Mentha arvensis L)).

The juice is extracted from this aromatic medicinal plant and its name as “herbal juice (Cs, Mk, Ma -618)”\. It has been used therapeutically from years all around the world because of its healing properties. The juice from aromatic medicinal plant is high in chlorophyll, active enzymes, vitamins and other important nutrients like Iron [2].

The Chlorophyll present in aromatic medicinal plant and hemoglobin has similar structures except for the central moiety. This juice is extensively used for the treatment of nutritional deficiency diseases like Anemia, Thalassemia, Inflammatory Bowel diseases, etc [3].

According to the National Family Health Survey - 3, more than half of women (55 %) aged between 14 to 50 years are Anaemic. The concentration of haemoglobin decrease in the blood due to poor dietary habits that causes deficiency of iron, proteins, vitamins like vitamin C, vitamin B12 along with folic acid and it is refers as anemia.
Deficiency of the above nutrients can affect the production of red blood cells. Anaemia is a widespread public health problem which is associated with an increased risk of morbidity and mortality. India is among the countries with the highest prevalence of iron deficiency anaemia in the world [4].

An anaemic person experiences many symptoms due to the low haemoglobin levels and reduction of oxygen available in organs and tissue. General body weakness, frequent tiredness, and lowered resistance to disease is the main symptoms (cost) of anaemia. Mainly pregnant women’s are suffering from anaemia; it is lead to premature delivery and low birth weight. It is of concern in children since anaemia is associated with impaired mental and physical development. The primary method of Anaemia diagnosis is the haemoglobin testing [5].

Age is associated with the category of Anaemia, with older women being somewhat more likely to be moderately or severely anaemic than younger women. The rate of moderate-to-severe Anaemia (moderate and severe Anaemia combined) among women of age 30-50 is almost three times as high as among girls of age 14-25 [5].

Figure 2: Structure of Hemoglobin and Chlorophyll [6].

Anemia can be treated by including iron, folic acid, and vitamin B12; vitamin C rich foods in diet. These nutrients can also be supplemented as drugs or in any other form like herbal juice (Cs, Mk, Ma-618). [6]. Chlorophyll also known as green blood [3] — The porphyrin heads structures of chlorophyll and hemoglobin can be defined relationship between both of them. Hemoglobin and Chlorophyll have similar structures. The main difference is that hemoglobin is built around iron (Fe), whereas chlorophyll is built around magnesium, (Mg).

Transport oxygen from the lungs to other parts of the body is the primary function of hemoglobin. Hemoglobin is composed of four elements- carbon, hydrogen, oxygen and nitrogen. All four are organized around iron. Chlorophyll is composed of the same elements, which are organized around magnesium (figure 2). Therefore this juice is often referred to as ‘Green Blood’. Therefore herbal juice (Cs, Mk, Ma-618) helps to build blood and is therapeutically used as-

- It increases the red blood count [3].
- Adjutant therapy in hemolytic anaemia [3].
- Management of inflammatory bowel disease. It improves digestion and metabolism [3].
- Antiasthmatic and Antiallergic agent [3].
- Acts as detoxifying agent. It contains antioxidants which repair damaged cells and rejuvenates aging cells. It purifies the blood and cleanses the kidneys, liver and urinary tract [8].
- Anemia is the most common disorder of the blood is anemia which has different types that produced by variety of causes. Its symptoms go undetermined in many people [7].

A condition in which healthy red blood cells or hemoglobin lack in blood is known as anemia Hemoglobin stands as a major part of red blood cell and it binds to oxygen. Abnormality in red blood cells or hemoglobin cause insufficient transfer of oxygen in your body. So the organs aren’t getting of their need to perform properly.
Types of anemia [8]

Iron deficiency anemia - Iron deficiency anemia is a condition in which the body has too little iron in the blood stream (For Indians Recommended Dietary Allowances for Iron for Normal adult male is 17mg/dl and for normal adult female is 21mg/dl which changes during pregnancy and lactation) [9].

It is microcytic anemia in which size of RBC reduces from normal size. This form of anemia is more common in young people and in women before menopause. For this type of diseases different reason contribute like Blood loss from heavy periods, internal bleeding from the gastrointestinal tract, or donating too much blood or other reason that causes iron deficiency anemia is a poor dietary habits or from chronic intestinal diseases. Treatment is usually done by orally supplementing iron and dietary modifications [10].

Folic acid deficiency anemia - Lack of folic acid forms this type of anemia. The B group of vitamins that helps your body make red blood cells, if you don’t have enough red blood cells, you have anemia. Low levels of folic acid cause megaloblastic anemia with this condition, red blood cells are larger than normal.

The folic acid deficiency anemia is occurs by an insufficient intake of folic acid, usually folic acid found in green leafy vegetables or by the overcooking of the vegetables, fresh fruits, fortified cereals, yeast and meats. Drink a lot of alcohol can also be a contributing factor in this form of anemia. The folic acid is used more during pregnancy or in infancy, this disease can also manifest itself.

Pernicious anemia - Decrease of vitamin B12 in body it cause Pernicious anemia. This anemia usually affects people ages between the 50 and 60. The disease can be hereditary and autoimmune disorder in which the body lacks the activity to make enough healthy red blood cells (RBCs).

The body requires vitamin B-12 and a type of protein called intrinsic factor (IF) to make red blood cells. Vitamin B-12, or Cobalamin, is found in certain foods and medications. A person who is suffering from any autoimmune diseases is more likely to contract pernicious anemia. It is also macrocytic anemia [10].

Aplastic anemia - Aplastic anemia is caused by an lack or lessening of all three blood cell types (pancytopenia): red blood cells (anemia), white blood cells (leucopenia), and platelets (thrombocytopenia).

The blood forming tissue in the bone marrow is damaged through the injury. Because of this, the sufferer is unable to fight infection and is likely to be a heavy bleeder. Aplastic anemia occurs in who is with acute viral hepatitis. Sickle cell anemia - This is an inherited form of anemia. It results in an abnormality in the oxygen-carrying protein hemoglobin (hemoglobin S) found in red blood cells. Sickle cell anemia is a life threatening disease and there is no prevention. Complications include leg ulcers, shock, cerebral haemorrhage, and orthopaedic disorders.

Grading of anemia

WHO Grading of Anemia: according to level of hemoglobin in blood [11]:

Grade 1 (Mild Anemia): < 10 g/dl
Grade 2 (Moderate Anemia): 7-10 g/dl
Grade 3 (Severe Anemia): < 7 g/dl

Anemia is mainly diagnosed by hemoglobin level in blood. Hemoglobin concentration measurement is among the most commonly performed blood test, usually as part of a Complete Blood Count.

Results are reported in g/L, g/dL or mol/L. Normal levels are:

Women: 12.1 to 15.1 g/dL (121 to 151 g/L, or 7.51 to 9.37 mmol/L)
Pregnant women: 11 to 12 g/dL (110 to 120 g/L, or 6.83 to 7.45 mmol/L)

If the concentration is below normal, this is called Anemia [12].

OBJECTIVES

The present study was undertaken with the following objectives:

1. Estimation of nutritional status of women (aged 14 to 25 and 30 to 50 years) for the prevalence of anemia.
2. Therapeutic trials with aromatic medicinal plant juice (30 ml/day for 30 days) for the prevention and control of anemia.
3. Determine the impact of interference with aromatic medicinal plant juice on blood haemoglobin levels.

II. MATERIALS AND METHODS

Plant collection:

Healthy fresh green leaves of coriander (Coriandrum sativum), Curry leave (Murraya koenigii L), and Mint (Mentha arvensis L) collected from the farm land’s in and around, Waghadia.

Fresh leaves are cleaned under tap water and then with distilled water, now dried plant material was weighed about 1gm of leaves using electronic weighing balance and finely cut.

Extraction of chlorophyll

Chlorophyll was estimated as described by S. Sadasivan and A. Manickam (1991).”[13] Biochemical methods”. 1 g of finely cut fresh leaves was ground to a fine pulp with the addition of 20 ml of 80% acetone with a mortar and pestle. It was then 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 colourless. The mortar and pestle were 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 was red at 645, 663 and 652nm against the solvent (80% acetone) blank.
Selection of area and subjects:
The present study was carried out in the This is a cross-sectional case control study carried out at Parul Seva Ashram (PASM), Limda, Waghadia, Vadodara, Gujarat. The study was approved & oral informed consent was obtained from the participants and normal subjects, prior to study. The participants were form village Anandpura, Ta- Sankheda, Dist-Chhotaudepur, Gujarat, for the analysis of Hb before and after the treatment of herbal juice (Cs, Mk, Ma-618) in this study a total number of 20 female subjects were included. These subject were analyze for Hb before and after treatment with individual (Coriandrum sativum), Curry leave (Murraya koenigii L.), and Mint (Mentha arvensis L) and the mixture of herbal juice (Cs, Mk, Ma -618).

Study included a group of 20 individuals consisting of females subject with different age group ranging from (14-25) and (30-50) ethnic background from high school and Labourers, after the oral informed consent taken from the patient by communicating in local language, 2 ml of venous blood drawn from the antecubital vein with all antiseptic precautions, 2 ml in EDTA Vacuette for Hb. Blood samples will be centrifuged at 2000 rpm for 15 mins. All the tests were done using automated analyzer.

Intervention with herbal juice (Cs, Mk, Ma -618)
The intervention with herbal juice (Cs, Mk, Ma-618) had done with 20 subjects i.e. women of age14 to 25 and 30 to 50 years for one month. These 20 subjects listed as experimental group and were given 30 ml of herbal juice (Cs, Mk, Ma-618) everyday early in the morning for 30 days. Special care was taken that the subjects were not taking any other supplements or drugs for control and cure of anemia.

Supplementation
About 30 ml of herbal juice (Cs, Mk, Ma -618) can be given per day i.e. 1 serving of herbal juice (Cs, Mk, Ma -618) is about 30 ml pre pared from different aromatic medicinal plant. Usually it is taken empty stomach and therefore taken early morning. Therefore it was given to subjects with proper procedures and care.

After 1 month of intervention, biochemical estimation of blood hemoglobin level was done using automated analyzer. Results were calculated using statistical methods [14].

The absorbance readings of the extract of the samples against 80% acetone blank are presented in the table:

<table>
<thead>
<tr>
<th>S No</th>
<th>Sample</th>
<th>645nm</th>
<th>652nm</th>
<th>663nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Coriandrum sativum</td>
<td>2.123</td>
<td>2.077</td>
<td>2.132</td>
</tr>
<tr>
<td>2</td>
<td>Murraya koenigii</td>
<td>2.147</td>
<td>1.379</td>
<td>2.150</td>
</tr>
<tr>
<td>3</td>
<td>Mentha arvensis</td>
<td>2.149</td>
<td>1.935</td>
<td>2.151</td>
</tr>
</tbody>
</table>

Foot Note: Absorbance taken at different wave lengths of three leaf samples.

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”:

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

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

Milligram of total chlorophyll per gram of tissue = \[20.2(A_{645}) + 8.02(A_{663})\] \times V/(1000\times W)

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

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

Coriander

Chlorophyll a (mg) = [12.7(2.152) – 2.69(2.123)] \times 0.1 = 2.162 mg

Chlorophyll b (mg) = [22.9(2.123) – 4.68(2.152)] \times 0.1 = 3.855 mg

Total chlorophyll (mg) = [20.2(2.123) + 8.02(2.152)] \times 0.1 = 6.014 mg

Curry leaves

Chlorophyll a (mg) = [12.7(2.150) – 2.69(2.147)] \times 0.1 = 2.153 mg

Chlorophyll b (mg) = [22.9(2.147) – 4.68(2.150)] \times 0.1 = 3.910 mg

Total chlorophyll (mg) = [20.2(2.147) + 8.02(2.150)] \times 0.1 = 6.061 mg

Mint leaves

Chlorophyll a (mg) = [12.7(2.151) – 2.69(2.149)] \times 0.1 = 2.154 mg

Chlorophyll b (mg) = [22.9(2.149) – 4.68(2.151)] \times 0.1 = 3.915 mg

Total chlorophyll (mg) = [20.2(2.149) + 8.02(2.151)] \times 0.1 = 6.066 mg

Statistical Analysis:
The results obtained were statistically analyzed by using continuous variables were presented as mean with standard deviations and then compared between different group of the study by applying Independent ’t ’ test. The results were expressed as mean± SD and were taken as significant when the probability (p<0.05), (p<0.001) as percentage of the observing values of ‘t’ at a particular degree of freedom & Pearson’s correlation analysis was performed.
### III. RESULTS

The amount of chlorophyll a, chlorophyll b and total chlorophyll as per calculations are presented in the table below:

<table>
<thead>
<tr>
<th>No</th>
<th>Sample</th>
<th>Chlorophyll a (mg/ml)</th>
<th>Chlorophyll b (mg/ml)</th>
<th>Total chlorophyll (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Coriandrum sativum</td>
<td>2.162</td>
<td>3.855</td>
<td>6.014</td>
</tr>
<tr>
<td>2</td>
<td>Murraya koenigii</td>
<td>2.153</td>
<td>3.910</td>
<td>6.061</td>
</tr>
<tr>
<td>3</td>
<td>Mentha arvensis</td>
<td>2.154</td>
<td>3.915</td>
<td>6.066</td>
</tr>
</tbody>
</table>

Foot Note: After calculation of total chlorophyll content of the leaf samples.

<table>
<thead>
<tr>
<th>Sample intervention</th>
<th>Mean(g/dl)±SD</th>
<th>P value</th>
<th>Percentage (%)</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture of herbal juice (Cs, Mk, Ma-618)</td>
<td>9.5± 0.65115</td>
<td>0.3044</td>
<td>76</td>
<td>6.88</td>
</tr>
<tr>
<td>Hb level post intervention after 1 month</td>
<td>10.36± 1.62677</td>
<td>82.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Murraya koenigii (Mk)</em> juice</td>
<td>11.08± 0.66151</td>
<td>0.3003</td>
<td>88.64</td>
<td>1.92</td>
</tr>
<tr>
<td>Hb level post intervention after 1 month</td>
<td>11.32± 0.70823</td>
<td>90.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mentha arvensis (Ma)</em> juice</td>
<td>11.52± 0.75206</td>
<td>0.9643</td>
<td>92.16</td>
<td>00</td>
</tr>
<tr>
<td>Hb level post intervention after 1 month</td>
<td>11.52± 0.7520</td>
<td>92.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Coriandrum sativum (Cs)</em> juice</td>
<td>10.95± 0.81700</td>
<td>0.3942</td>
<td>87.6</td>
<td>4.64</td>
</tr>
<tr>
<td>Hb level post intervention after 1 month</td>
<td>11.53±1.17127</td>
<td>92.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Foot Note: pre and post intervention result shows increase in hemoglobin percentage all the subjects accept alone mint juice subjects.
IV. DISCUSSION

Chlorophyll estimation was done in the fresh green leaf samples extracted with the acetone solvent the absorbency readings of chlorophyll extracts were measured in three different wavelengths 645nm, 663nm and 652nm respectively. Based on the absorbency value calculations were made using S. Sadasivan and A.Manickam (1991) equations and the amount of chlorophyll a, chlorophyll b and total chlorophyll were estimated and tabulated. According to Rajalakshmi.k et. al (2015) all the medicinal plant used in the study were analysed and Mimosa Pudica show highest chlorophyll content at the same time some of the antinutritiona factors enable to digest. But our study used plants are kitchen plants full utilized and digested without any interfering health condition of the subjects.

In our study it was found that Mint, Curry, Coriander leaves have the almost same and sufficient amount of chlorophyll content. 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. 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. So this different aromatic medicinal plant leaves can be use for the treatment of anaemia. For treatment of anaemia about 30 ml of herbal juice (Cs, Mk, Ma- 618) can be given per day and prepared from different aromatic medicinal plant. Usually it is taken empty stomach and therefore taken early morning. Therefore it was given to subjects with proper procedures and care.

After 1 month of intervention, biochemical estimation of blood haemoglobin level was done using automated analyser. Results were calculated using statistical methods [14].

Table 3 depicts that Pre Intervention the mean haemoglobin level of the different- subjects of different groups was 9.5 .11.08,11.52 and 10.95 g/dl and Post Intervention it was found to be increased to about 10.36,11.34,11.5,11.52 g/dl. There was an about 0.86,0.26,0.0,0.58 g/dl respectively increase in haemoglobin level of blood.

These shows the 3 group of them give a positive impact of intervention with aromatic medicinal plant leaves juice for treatment of anemia and one group shows no effect of this juice.

Those 17 subjects from the group didn’t complain of any side effects of wheat grass juice. Only 1 subject complained about head ache at the beginning of intervention and only 1 complained about stomach ache also at the beginning of intervention. The reason for headache was that the aromatic medicinal plant leaves is known to have a cleansing effect on the body. When our bodies become accustomed to certain stimulants such as sugar or caffeine, the cleansing process can eliminate those stimulants from our bodies. Since the brain becomes used to having these types of pick-me-ups and suddenly stops receiving them, it often results in a headache. Also the reason for stomach ache was that the juice helps in relieving constipation.

We are observed a case in our study age group of (30-50) women have some health problem related to physiological anemia. Subject hemoglobin level is 10.3 g/dl. She taken unani treatment but still she did not get positive result; there is no much change in her hemoglobin percentage. Same subject after use of our herbal juice (Cs, Mk, Ma -618) for 1 month treatment she got positive result increase in her hemoglobin is 10.6 g/dl, in a month (30 days) she reduce her patches on her face. So she is continuing drinking the juice till another two months after that her face patches are totally cleared and her iron levels also increased well without any other side effects.

V. CONCLUSION

After estimating the chlorophyll content of (Coriandrum sativum), Curry leave (Murraya koenigii L), and Mint (Mentha arvensis L) by using spectrophotometric methods using acetone as the solvent, it was found out that all of three have almost same content of chlorophyll, thus these leaves can be utilized as therapeutic agents for treatment of various problems including anemia, thalassemia, etc. They are also easily available and inexpensive.

The use of herbal juice (Cs, Mk, Ma -618) for increasing the blood hemoglobin level for treating anemia. The beneficial effects of different aromatic medicinal plant juice have been established by studies done with subjects.

The findings revealed that the subjects coming under the age group 14 to 25 and 30 to 50 years were moderately anemic. The study about the impact of intervention with herbal juice (Cs, Mk, Ma -618) on blood hemoglobin level revealed that some subjects found an increase in their hemoglobin level of blood and some subject decrease in their hemoglobin level of blood. This was proved with the help of biochemical estimation at the beginning and end of intervention. The beginning the mean hemoglobin level of the subjects was gradually increase.

This shows that there is a positive effect of herbal juice (Cs, Mk, Ma -618) on blood hemoglobin level i.e. the hemoglobin level of subjects raised after consumption of juice for 30 days. Therefore herbal juice (Cs, Mk, Ma -618) proved to be useful for the treatment of any kind nutritional deficiency of anemia.

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