COMPARATIVE STUDY ON THE PROXIMATE ANALYSIS OF MEDICinally
IMPORTANT PLANTS (NEEM AND BUTTERFLY PEA PLANT)

Jennica Rose¹, Autif Hussain Mangi¹* and Fahim Ullah Khan²

¹ Institute of Biochemistry, University of Sindh Jamshoro, Sindh, Pakistan
² Department of Zoology, University of Science and Technology Bannu, Khyber Pakhtunkhwa, Pakistan
*Corresponding Author Email: Dr. Autif Hussain Mangi (autif@usindh.edu.pk)

ABSTRACT: The Neem and Butterfly Pea Plants have gained much attention recently due to their medicinal value, therapeutic effect, and nutritive role. A comparative study of Neem and Butterfly Pea Plants through biochemical parameters proves helpful in determining their role in a preferential manner. This was an analytic/experimental study on the proximate analysis of Neem and Butterfly pea plant. The plant sampling was done within the premises of the University of Sindh in the month of December 2022. The proximate analysis was done in the lab of the Institute of Biochemistry University of Sindh. The resulting data was compiled and the average and standard deviation were calculated from the replicate values of Neem and Butterfly pea plant samples. Further data was analyzed using SPSS software version 21, and a t-test was performed to find the level of significance between the two groups. The P value of <0.05 was set as statistically significant. We observed that the Butterfly pea plants were more under the effect of Nitric acid and Ethanol compared to the Neem. Also, the Leaves and Stem obtained from the Butterfly pea plants were more under effect of Nitric acid and Ethanol compared to the Neem. 88% of the stem portion of Butterfly Pea Plant was solubilized by HNO₃ and 92% of the leaf portion of Butterfly Pea Plant was solubilized by HNO₃. The results from our proximate analysis indicated that the proximate values Butterfly Pea Plants vary greatly compared to the Neem. Our results concluded that the Butterfly pea plants had more ethanol soluble and Nitric acid soluble components compared to the Neem. This study is important in providing additional knowledge about proximate values, the medicinal and biochemical role of Butterfly Pea Plants, and their comparison with the Neem.

Index Terms- Butterfly pea, Comparative study, Neem, Proximate analysis.

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INTRODUCTION

Miracle tree Neem with numerous benefits for humans has been considered of great pharmacological values for centuries. On the other hand, Butterfly pea, or Clitoria ternatea, is a perennial herbaceous plant that belongs to the Fabaceae family. The Neem with the botanical word “Azadirachta Indica”, is derived from Persian words i-e; “Azad”- free and "Dirakht" tree according to some sources [Venugopalan Santosh et al, 2013]. The Neem tree is an incredible plant that has been declared "Tree of 21st Century" by the UN (United Nation). The family of the Neem tree is Meliaceae, and the most beneficial and pharmacologically important for the health and benefit of mankind. Neem can reach 15-30m (49-98 feet) in height and have adorable rounded crowns and thick furrowed bark [Melissa Detruzzelo, 2023]. Compound leaves have toothed leaflets and are typically evergreen but drop during periods of drought. Indeed, the “Village Pharmacy”-Neem tree, can live for about 150-200 years. Azadirachta indica leaves aquatic or methanolic extract is effective against snake or scorpion envenomation [Nadia M.S et al, 2017]. Neem flower possesses stronger hypoglycemic activity than the bark when extracts were administered in streptozotocin-induced diabetic mice. [Ashok Purohit and V.P.Dixit, 1991]. In glucose tolerance test in diabetic rats with Neem extract at the dose of 250 mg/kg demonstrated that glucose levels were significantly less compared to the control group. The Butterfly pea plant has recently gained a lot of attention because it has the potential to be used in agriculture, modern medicine, and as a source of natural food colorants. Antioxidants, anti-diabetes, anti-obesity, anti-inflammatory, anti-cancer, anti-hyperlipidemia, anti-asthma, and other health benefits are just a few. Drinks containing butterfly peas have recently gained popularity due to the butterfly pea flower's well-known health benefit According to research study of [Piya Kosai et al., 2015], for the treatment of "Masasika roga" (mental illness), Clitoria ternatea commonly known as Butterfly pea plant, proved to be a much better "Medhya"(toning the brain) drug and is a highly valued herb as it acts as a rejuvenator for brain and nervous system.

Plants with medicinal properties are an important source for drug industries. The medicines directly derived and formed from the organic and natural plant based products are far better than the medicines formulated by artificial synthesizers. Currently, more research is going on to discover new plants with medicinal value and also to
compare the medicinal properties of already discovered plants. The medicinal characteristics of Neem have been thoroughly studied, however, there is not enough research available regarding the comparative study of Neem with other Plants. The butterfly pea plant has recently gained much importance regarding it’s role in human health. Thus it will be of great significance to compare the proximate characteristics of Neem and Butterfly Pea Plant.

RESEARCH METHODOLOGY

Study site and sampling: This comparative study was conducted on the proximate analysis of two nature's drugstore plants named the Neem tree and the Butterfly pea plant. The samples were collected from Neem tree and Butterfly Pea plant which were two different location premises. The study site had also many other types of plants and trees. The study site had some rocky, concentrated, granular, and loamy soil portion. The study site had a mostly dry surface in the case of Neem tree whereas, the butterfly Pea plant had some loamy soil surface.

The fresh samples of leaves and stems of Neem tree were collected from the Kotri site area. The Butterfly Pea plants were purchased from the Sindh Agriculture University, Tandojam. Moreover, we took care of the Butterfly Pea plant in the garden of the Institute of Biochemistry, University of Sindh (Figure 1).

Analysis of Total Ash from Neem and Butterfly Pea Plant: About 600g of our samples (leaves and stems) of Neem and Butterfly Pea plant, after drying in the oven we burnt the samples of leaves and stems into ash. 600g of each plant Neem and Butterfly pea plant samples of leaves and stems were put in the crucible. We applied all standard methods for the analysis of Ash that included the Muffle furnace method, microwave ashing method, and crucible method [Nilima et al., 2011]. We here mention the crucible method experiment as it was very easy to conduct inside the laboratory and it provided interesting results during comparison between Neem and Butterfly Pea Plants.

Treatment with water solvent: 5g ash of leaves and 5g of stems along with 5ml of distilled water in each, were separately poured into 5 test tubes and set the rack of sample test tubes in the oven for about one hour at 100o centigrade. After that, we filter the solution of ash and water using percolate paper and separate the extractive from the insolubilized ash. We weigh the percolate and residue both using analytical weighing balance.

Treatment with Nitric Acid: The weight of ash of leaves 5g and 5g stems coupled individually with 5ml of concentrated Nitric acid (HNO₃) were shifted into five test tubes replicates and placed on the shelf of laboratory for an hour, then filtered using filter paper, and percolate was collected in the conical flask and residue remained on the
filter paper. Percolate and residue were weighed again using an electronic analytical weighing balance machine.

**Treatment with water Ethanol:** Ash of weight 5g leaves and 5g of ash stems along with 5ml of ethanol were set in replicates of five test tubes, the test tubes were left in the laboratory for an hour, and then, percolate and residues were filtered using filter paper and in the end, the percolate and residue were weight using Analytic balance.

**RESULTS**

Our research results showed that the ash obtained from the Butterfly Pea plants was more under the effect of Nitric acid and Ethanol compared to the Neem. Leaf portions of both plants were greater under the effect of Ethanol such as 84% of Neem Leaf Filtrate and 86% of Butterfly Pea Plant Leaf Filtrate. The ash from the leaves of two plants showed decreased solubility when treated with water (Figure 2).

As far as the treatment with Nitric acid (HNO₃) was concerned, we found that it solubilized ash of Neem and Butterfly Pea plant leaves quite well but it had more solubility when made a solution with Butterfly pea plant leaves in contrast to Neem leaves (Table 1).

![Figure 2: Effect of solvents on Neem and Butterfly pea plant leaves](image)

**Table 1: Solvent effect on stem portion of Neem and Butterfly pea plant.**

<table>
<thead>
<tr>
<th>Solvents (n=3)</th>
<th>Neem (gms)</th>
<th>Butterfly pea (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (H₂O)</td>
<td>3.17</td>
<td>3.42</td>
</tr>
<tr>
<td>Nitric acid (HNO₃)</td>
<td>4.23</td>
<td>6.20</td>
</tr>
<tr>
<td>Ethanol (C₂H₅O)</td>
<td>2.60</td>
<td>3.60</td>
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</tbody>
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We also compared the ash values of Neem and Butterfly pea plant stems when solubilized with solvents. We observed that both of the plants showed great variability when solubilized with Ethanol as the values were 83% and 92% respectively but the Neem stem had less solubility such as 78% when treated with water and 67% after treatment with Nitric acid as compared to the stems filtrate of Butterfly pea plant which was 80% with water and 88% with Nitric acid respectively (Figure 3).

After comparing the sections of leaves and stems of both plants (Figure 3), we found out that the ash obtained from the Butterfly pea plant was more under the effect of Nitric acid and Ethanol compared to the Neem. Both stem and leaf portion of the Butterfly pea plant were greater under the effect of Nitric acid and Ethanol such as
88%, 92%, and 87%, and 86% respectively. Our research results suggested that Ethanol was an efficient solvent regarding its effect on Butterfly Pea Plant (Figure 3). The results also showed that the Butterfly Pea plant was solubilized more easily and quickly than Neem (Table 1).

Table 2: Solvent effect on leaf portion of Neem and Butterfly pea plant.

<table>
<thead>
<tr>
<th>Solvents (n=3)</th>
<th>Neem (gms)</th>
<th>Butterfly pea (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (H_{2}O)</td>
<td>3.46</td>
<td>2.82</td>
</tr>
<tr>
<td>Nitric acid (HNO_{3})</td>
<td>3.69</td>
<td>5.41</td>
</tr>
<tr>
<td>Ethanol (C_{2}H_{4}O)</td>
<td>2.93</td>
<td>3.17</td>
</tr>
</tbody>
</table>

The leaf portion of both the plants had a great effect on solubility when the ash of leaves of Neem and Butterfly pea plant solubilized with Nitric acid (Figure 3), as compared to Butterfly pea plant leaves (Table 2), while the other two solvents showed lower effect when treated with the ash of both the plants. After ash was made from Neem (stem) and Butterfly pea plant (stem) manually by using wooden match sticks, we measured the ash of both plants stems using the weight machine and equally divided the ash into five replicates of both the plants (Figure 2). The calculated values showed that the ash content of butterfly pea stem was higher than the ash content of Neem (Table 2).

DISCUSSION

In our research work, we found two main reasons behind the varying proximate values of Butterfly Pea Plants and Neem. One reason was the method of analysis and the other reason was the mode of analysis (comparative analysis). We observed that when we treated Neem and Butterfly pea plant leaves with three different solvents; water, nitric acid, and ethanol, the outcomes demonstrated that ethanol was a quite better solvent and also we gained support for our work from previous research that reported an excellent solvent effect of Etanol as it solubilized flavonoids, saponins in the Neem leaves (Zainab et al., 2016), and their results were comparable and consistent to our results. Another research similarly reported about filtrate anthocyanin, and glycosides from the leaves of Butterfly pea (Ethel Jeyaseela Jeyaraj et al., 2021) which were in accordance with our calculation. During our research work the water also showed good solubilizing ability as 78% of the filtrate was in both the cases of Neem and Butterfly pea plant. However, following similar research we understood that the Nitric acid solubilized some of the metals present in Neem leaves like Ca, Fe, Mn, Cd (Ideisam I Abu-Abdoun et al., 2019) and Butterfly pea had saponin (Edema et al., 2016) content which was soluble in nitric acid (Jamie Love et al., 2020). In our research work, we found that the Neem stem samples treated with the ethanol showed interesting variations in results. There could be chances that Neem stem samples contained some of the ingredients having a lipid nature that’s why the ethanol proved to be a good solvent for solubilizing them. According to some previous research work done on the Neem stem, it was shown that ethanol dissolves polysaccharide and Neem stem contain polysaccharide (Xun Yan et al., 2017). Butterfly stem samples treated with the ethanol also showed interesting results as we observed that the values of filtrate were higher than the residues which exhibited a magnificent role of ethanol. The previous research findings have kept supporting our current research work as they mentioned the role of ethanol in dissolving phenolic compounds also (Joana Schuelter Boeing et al., 2014), and the butterfly stem also
have phenolic compounds (Min shi et al., 2003). Neem contained some metals also [Joseph Clement Akan et al., 2012] and the results evidently revealed that the Butterfly pea plant stem values were greater than Neem Stem values. It may be due to the reason that as we burnt the stems manually by using match sticks and maybe some of the residues of wooden sticks may be the part of the ash content. Another reason maybe some of the organic matter of the stem that could not be burnt completely. After observing the physical appearance of both plants we understood that the textural characteristics could also be one of the reasons because the Butterfly pea plant stems were soft in nature with lesser presence of woody content that were grown in the upward direction. On the other hand, the Neem stem had a woody texture that took time to burn into the ashes. As we had already observed that the butterfly pea leaves had a bit higher value of ash content when compared with the Neem leaves. It was determined very clearly that the Butterfly pea plant leaves value was higher than Neem leaves and the reason could be the presence of some organic matter from leaves that could not be burnt completely. We have also observed in our research that almost all parts of the Neem including leaves and stem and full parts of Butterfly pea plant showed significant effects when they were treated with the Nitric acid however, the Neem showed smaller effect when treated with nitric acid comparatively to the Butterfly pea plant. In this way, our work reflected that the Butterfly pea plant may contain more soluble metallic compounds as compared to the Neem.

Conclusion: Our research work concluded that the comparative study between the Neem and Butterfly Pea Plant was important in obtaining additional knowledge about the varying proximate values between the two plants. Our research study also provided information about the efficacy of solvents when treated with Neem and Butterfly Pea Plants. Our research work also gave an idea about using the crucible method as an effective way to quickly and authentically conduct proximate analysis of Plants. The research work we had conducted, on the comparative proximate analysis of two natures extraordinary plants Neem Tree and Butterfly Pea Plant showed noticeable differences when treated with three dissimilar solvents; Water, Ethanol, and concentrated Nitric acid. The solution of ash and three solvents with separate treatment exhibited that the percolate of both the plants, Neem, and Butterfly Pea were higher as compared to the residues. After comparison, we found that the Neem stem had higher values of percolate as compared to the Butterfly pea plant stem and the values of percolate of Butterfly pea plant leaves were higher as compared to Neem leaves. Our results and observations were quite meaningful and our study seemed to conclude with achieving our main targets and objectives which were the comparative study of the two plants Neem and Butterfly Pea. Our work was useful in finding the comparison between Neem and Butterfly pea plant as we found that the Butterfly pea plants had more ethanol soluble and Nitric acid soluble components compared to the Neem. The quantity of percolate of both the plants (Neem and butterfly) was higher as compared to the residues. The comparative study on the proximate analysis of Neem and Butterfly pea plant provided knowledgeable and meaningful insights. Further research focus is required on the biochemical and medicinal role of the Butterfly Pea Plant.

Conflict Of Interest: There is no conflict of interest to declare

Author’s Contribution:
Conceptualization and Methodology: Autif Hussain Mangi, Jennica Rose
Investigation: Fahim Ullah Khan, Autif Hussain Mangi
Formal Analysis: Autif Hussain Mangi, Jennica Rose
Writing Original Draft: Jennica Rose, Autif Hussain
Writing review, editing, and Supervision: Fahim Ullah Khan, Autif Hussain Mangi

REFERENCES
Patil, P., Patil, S., Mane, A., & Verma, S. (2013). Antidiabetic activity of alcoholic extract of neem...

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