

Effect of Environmental Conditions on Soluble Proteins of *Ailanthus excelsa*'s Leaves

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S.Em.-Standard error of the mean

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Abstract

The tree of *Ailanthus excelsa* Roxb. is a good source for various secondary metabolites as tannins, saponins,

glycosides, triterpenes etc. The leaves of *Ailanthus excelsa* Roxb. is a good source of proteins. The quantities of soluble proteins were determined by Lowry method and the percent quantities of proteins from leaves of *Ailanthus excelsa* Roxb. at Mandsaur, District Mandsaur and Ratlam, District Ratlam (Fig. 4) had varied. The percentage of quantities of Mandsaur, District Mandsaur were 5.7748%, 5.800%, 5.8125%, 5.7874%, and 5.8251% while Ratlam, District Ratlam had 4.7251%, 4.7375%, 4.7500%, 4.7748% and 4.7624%. The S.Em. value was 0.432705 and CD 5% value was 1.27648. The data showed the significant value. Thus, the leaves of *Ailanthus excelsa* are valuable source of proteins.

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1. Introduction

The medicinal plants are the main source of various effective contents that are primary and secondary metabolites. Each part of medicinal plant is useful for the treatment of diseases since ancient times. The Ayurvedic medicinal contents of plants are very useful, safe with no side effects compared to allopathic medicines (Okigbo et al. 2008). The proteins are the primary metabolic macromolecules that are compulsory parts for humans and animals. The proteins have high molecular weight and the amino acid sequences are present in it which was arranged in linear order.

1.1 Introduction of Selected Plant

The *Ailanthus excelsa* Roxb. is a tree of high height. Its leaves shape is similar to *Azadirachta indica* (Neem) therefore *Ailanthus excelsa*'s common name is Mahaneem. Three protein fractions respectively unfractionated, chloroplastic and cytoplasmic were present in fresh leaves of *A. excelsa*. The cytoplasmic protein fraction contained 62.71% crude protein, while whole leaf contained 20.86%. The unfractionated and chloroplastic

protein fractions contained more crude fat than the whole leaf and the pressed cake. The protein fractions were very low in crude fiber (Nag et al., 1994). Most of the amino acids were found in the leaf and their composition of amino acids is excellent (Lavhale et al. 2007). The seed protein concentration was found to be 14 and 8%. (Kundu et al., 2009).

2. Objective of Research

The main object of this research was to know the effect of environmental conditions on same plant species that grows in different places.

3. Justification of Research

This research is very useful for human and also for cattle because various metabolic contents can increase by the effect of environmental conditions. The increased quantities of metabolites can be used in the treatment of human diseases.

4. Material and Methods

Summary of Work Plan

The leaves were collected from five different places of Mandsaur (Madhya Pradesh) and Ratlam (Madhya Pradesh) and shaded dried. The soluble proteins were extracted and quantity of soluble proteins were determined with standard protein curve.

Collection of Plant Materials

The fresh leaves of *Ailanthus excelsa* Roxb. (fig. 3) belonging to the family Simaroubaceae, were collected from five different places in Mandsaur, districts Mandsaur, Madhya Pradesh and Ratlam District Ratlam, Madhya Pradesh (fig. 1). The places of Mandsaur were indicated by M-1, M-2, M-3, M-4 and M-5. The places of Ratlam were indicated by R-1, R-2, R-3, R-4 and R-5. The District Mandsaur is situated at the northern projection of Madhya Pradesh between the parallels of latitude $23^{\circ} 45' 50''$ North and $25^{\circ} 2' 55''$ North, between the meridians of longitude $74^{\circ} 42' 30''$ East and $75^{\circ} 50' 20''$ East. The District Ratlam is situated in North-West region of Madhya Pradesh from $23^{\circ} 05'$ North to $23^{\circ} 52'$ North longitude and $74^{\circ} 31'$ East to $75^{\circ} 41'$ East latitude. The plant material was taxonomically identified, confirmed and authenticated by Dr. Rakesh Mohan Painuli, Incharge, Herbarium, Department of Botany, HNB Garhwal University, Srinagar (Garhwal), Uttarakhand The identification code is (HNB 20705). The collected green leaves (fig. 2) were shade dried and then the dried material was crushed to coarse powder in grinder. The powder was stored in an airtight container for extraction.

Figure 1: Map of Madhya Pradesh



Figure 2: Photograph of *Ailanthus excelsa* Roxb. Plant



Figure 3: Photograph of *Ailanthus excelsa* Roxb. leaf collected from Mandsaur District Mandsaur (Madhya Pradesh)



Extraction and Estimation of Protein from Leaves of *Ailanthus excelsa* Roxb.

Proteins Extraction

The shade dried leaves of *Ailanthus excelsa* were homogenized separately in 10% cold trichloroacetic acid (10 mg: 5ml). After samples' homogenization, it was centrifuged at 5000 rpm for 10 minutes. After centrifugation method, two layers were generated in which the supernatant layer was discarded and pellets were saved. These pellets were again suspended in 5 ml of 10% cold TCA (Merck) and recentrifuged for 10 minutes. The supernatant and precipitate of leaves were found. After the recentrifugation method, the supernatant part was discarded and the precipitate part was dissolved into 10 ml. of 0.1 N NaOH. After dissolution process of precipitation, 0.1 ml of this solution was used for protein estimation.

Quantitative Estimation of Proteins

The total protein content was estimated using the protocol of Lowry et al., 1951. A stock solution (1mg/ml) of bovine serum albumin was prepared in 1N NaOH. Then, prepared different concentrations (0.2, 0.4, 0.6, 0.8 and 1ml) of BSA from the working standard solution. They were taken in a series of test tubes and in another set of test tubes, 0.1 ml and 0.2 ml of the extracted samples were taken. After it, the volume was raised upto 1 ml in all the test tubes and 2 ml freshly prepared alkaline solution (prepared by mixing 50 ml of 2% Na₂CO₃ in 0.1 N NaOH and 1 ml of 0.5% CuSO₄.5H₂O in 1% sodium potassium tartrate) was added in each test tube at the room temperature. They were left for 20 minutes incubation at the room temperature without any disturbance. After 20 minutes incubation period, 0.2 ml Folin-ciocaltau reagent (diluted with equal volume of distilled water just before use) (Merck) was rapidly added, mixed by vortex mixture and incubated at the room temperature (about 25°C) for 30 minutes until the blue color developed. The optical density was recorded on spectrophotometer (Systonic). It was adjusted at wavelength of 750 nm and set at 100% transmittance using blank before taking the readings of the standard and the test samples respectively. A standard curve was prepared with various concentrations of the standard solution series against their respective absorbances, following the Lambert-Beer's law (Talreja, 2011).

5. Result

Proteins are one of the most important parts of leaves which provide genetic information. The leaves of *Ailanthus excelsa* were collected from different places where the climatic conditions differed. The temperature and climatic conditions have morphological effects and also have genetic changes. The different quantities of proteins in the equal amount of dry leaves powder, proved the genetic variations in the same plant species. The plant's leaves were collected from Mandasaur and Ratlam (M.P.) and thereafter dried in shade. The protein's quantity was extracted from dried leaves with the help of perchloric acid and TCA solution. The proteins' concentrations were calculated with the help of standard curve of bovine serum albumin that is a standard protein and measured at 750 nm.

Figure 4: Comparative Study of (%) Soluble Protein in *Ailanthus excelsa* Roxb

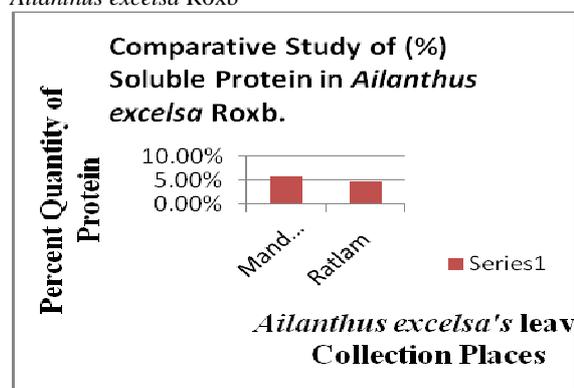


Table 1: Quantitative Estimation of Standard Bovine Serum Albumin Protein

Sr. No.	BSA (ml.) (100mg/100ml)	Distilled Water (ml.)	Sample Conc.	Reagent- C (ml.)	Folin-Ciocalteu Reagent (ml.)	O.D. (750 nm)
01	Blank	2.0	0.00	2.0	0.2	0.000
02	0.1	1.9	0.02	2.0	0.2	0.089
03	0.2	1.8	0.04	2.0	0.2	0.184
04	0.3	1.7	0.06	2.0	0.2	0.260
05	0.4	1.6	0.08	2.0	0.2	0.357
06	0.5	1.5	0.10	2.0	0.2	0.402
07	0.6	1.4	0.12	2.0	0.2	0.503
08	0.7	1.3	0.14	2.0	0.2	0.552
09	0.8	1.2	0.16	2.0	0.2	0.609
10	0.9	1.1	0.18	2.0	0.2	0.679

Table 2: The Comparative Percent Quantities of Soluble Proteins in Leaves of Mandasaur and Ratlam (M.P.)

Replications	% Protein of Mandasaur	% Protein of Ratlam
R1	5.7748	4.7251
R2	5.800	4.7375
R3	5.8125	4.7500
R4	5.7874	4.7748
R5	5.8251	4.7624
Mean	5.800	4.750
S.E.m.	0.432705	
CD 5%	1.27648	

The percentage quantities of protein of five replicates, collected from Mandsaur (M.P.) and Ratlam, were mentioned in table 2. The % quantities of protein of five replicates from District Mandsaur (Madhya Pradesh) were 5.7748, 5.800, 5.8125, 5.7874, 5.8251 and their mean value was 5.800%. The % quantities of protein of five replicates from District Ratlam (Madhya Pradesh) were found to be 4.7251, 4.7375, 4.7500, 4.7748, 4.7624 and their mean value was 4.750%. The replicate datas were calculated by two ways ANOVA and compared with curve (fig. 4)

Discussion

The five different samples were collected from Mandsaur (M.P.) and Ratlam (M.P.) and soluble proteins' quantity were calculated with standard bovine serum albumin protein curve. The environmental conditions are very effective on plant and human life. Climatic conditions were also effective on morphological aspect of plant leaf shape (Kumar et al. 2010). The protein quantity was increased in Mandsaur (M.P.) compared to Ratlam (M.P.). The environmental conditions of Mandsaur (M.P.) very differ from Ratlam (M.P.).

Conclusion

Various secondary effective metabolites such as tannins, saponins, phenols, glycosides etc. are present in the leaves of *Ailanthus excelsa* Roxb. (Bhatt S. et al. 2012). Secondary and primary metabolites are present in more quantity. Therefore, the leaf of *Ailanthus excelsa* is a useful source of protein and a good source for cattle as a fodder.

The environmental conditions and variety of soils effect the quantity of primary and secondary metabolites. In the comparative study of proteins' quantity, Mandsaur, District Mandsaur and Ratlam, District Ratlam had different quantity of proteins. The main reason is of environmental conditions, situations and soil. Therefore, the quantity varies in different environment.

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