International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395 -0056
Volume: 04 Issue: 06 | June -2017 www.irjet.net p-ISSN: 2395-0072

Lab scale extraction of mimosine from leucaena leucocephala leaves

Ghanshyam Pund[1], Dr.D.C. Kothari[2], Dr.P.V.Thorat[3]

[1]*M.Tech (II Year), Chemical Engg. College of Engineering & Technology, Akola (M.S), India-444104*
[2] *Professor, Chemical Engg. College of Engineering & Technology, Akola (M.S), India-444104*
[3] *HOD, Chemical Engineering Dept., College of Engineering & Technology, Akola (M.S), India-444104*

Abstract - *Mimosine [α-amino-β-(3-hydroxy-4-oxo-1, 4-dihydropyridin-1-yl)-propanoic acid] is toxic element, that found naturally in Leucaena (Leucaena-leucocephala de Wit) tree.*

Leucaena-leucocephala de Wit is a popular farm forestry tree may resolve the shortage of animal feeds, but usage is limited due to the presence of a toxic non-protein amino acid, mimosine in this plants, which results in growth retardation, cataracts and infertility in animals. This toxicological nature of the mimosine is also responsible for the herbicidal activity of mimosine towards the some weeds.

The findings of this research is to explore the lab scale extraction process of mimosine from Leucaena-leucocephala de Wit and to review the herbicidal nature of mimosine.

Key Words: Mimosine, Leucaena-leucocephala de Wit, Toxicological, Extraction, Pesticidal Nature.

1. INTRODUCTION

Leucaena-leucocephala de Wit called "Subabul" in India is a popular farm forestry tree in the coastal areas of Andhra Pradesh. It is estimated that in Prakasam district of Andhra Pradesh alone. Leucaena-leucocephala is planted in area exceeding 50,000 ha. It is one of the fast growing hardy evergreen species in India. It is a vigorous coppiced and responds well to pollarding, lopping & pruning. It has deep and strong taproot and even the seedlings are deep rooted. There are four types of Leucaena-leucocephala. [2]

1.1 Types of Leucaena-Leucocephala Plant

- **Hawaiian Type:** The plants are short bushy and remarkably drought tolerant. It is suited to hilly terrains in drought prone areas. It is a prolific seed producer and is good for fodder purpose
- **Salvador Type:** Tall, tree like and fast growing having maximum annual biomass production. Possesses large leaves, pods and seeds than Hawaiian types. Responds to high fertilization.
- **Peru Type:** Tall and extensively branching type and is ideal for fodder purpose.
- **Cunningham Type:** It is a cross between Salvador and Peru types.

Leucaena-leuc0ocephala is best suited for warm regions and grows well between 22 and 30ºC in regions of 500 to 2000 mm annual rainfall. Because of its strong and deep root system, the tree is highly drought resistant. [2]

Fig -1: Leucaena-leucocephala de wit (subabul).

1.2 Mimosine

Mimosine [β-[N-(3-hydroxy-4-oxypyridyl)]-α-amino-propionic acid] is a non-protein amino acid, and is a major compound present in all plant parts of mimosaceae, which includes Leucaena (Leucaena-leucocephala), Leucaena-glauca, and other legumes belonging to Mimosa spp. Structurally, mimosine is an analog of di-hydroxy-phenylalanine with a 3-hydroxy-4-pyridone ring instead of a 3,4-dihydroxy-phenyl ring (Fig-2).

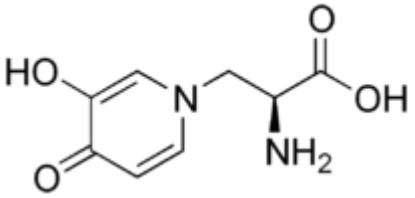

Fig-2:(2*S*)-2-Amino-3-(3-hydroxy-4-oxopyridin-1-yl) propanoic acid.

Although Leucaena has a rich protein content and high annual yield, the presence of mimosine has limited the wide use of this plant as animal feed. This compound causes alopecia, growth retardation, cataracts and infertility in animals. Mimosine possesses antimitotic activity that blocks the cell cycle in the large G1 phase and inhibits DNA synthesis, which prevents the formation of the replication fork by altering deoxy-ribo-nucleotide metabolism [3]. Mimosine has structural formula C8H10N2O4 and molecular wt .198.18 mol^{-1} with melting point 291ºC

1.3 Discovery of Mimosine

Mimosine was first isolated from the sap of Mimosa pudica by Renz and was given the name "mimosine". Later, minosine was biologically characterized from M. pudica by Nienburg and Taubõck from the extraction of ground Leucaena-glauca seeds. The chemical structure of mimosine was determined by Bickel as β-N-(3-hydroxy-4pyridone)-α-amino propionic acid. The structure of mimosine is similar to di-hydroxy-phenylalanine with a 3-hydroxy-4-pyridone ring instead of a 3, 4-dihydroxy-phenyl ring [3].

1.4 Herbicidal Activity of Mimosine

Mimosine has long been known as an allelochemical produced by Leucaena, and the injury this legume caused the other plants in its surroundings is attributed to toxicity similar to mimosine. Mimosine may enter soil through the Leucaena root, and the leaves decomposition. However, Leucaena grows in subtropical and tropical areas, which commonly have a great annual rainfall that may also lead to the exudates of mimosine from Leucaena plant parts to the soil. This contributes to the phenomenon of soil injury.

Some experimental studies (Table-1) shows that mimosine appeared to have a strong suppression of the length of radicles and hypocotyls of the six tested plants, although at the doses 1 and 10 mg/L, mimosine promoted emergence of the six tested plants, except for P. vulgaris growth, which was inhibited by 30–40%. At 50–1000 mg/L, these indicator plants were strongly stunted by mimosine, and the growth of B. Rapa was almost completely inhibited.

At a concentration up to 100 mg/L, mimosine did not show any inhibition of the length of radicles and hypocotyls of Leucaena, while it displayed a strong suppression of the emergence of other plants. Besides Leucaena, M. pudica is also a mimosine producer therefore mimosine at a low concentration did not influence emergence of the two plants. Among the tested plants, B. pilosa and L. multiflorum were inhibited by mimosine at 25 mg/L, and the inhibition was proportional to the applied dose of this compound, suggesting that mimosine may be effectively exploited as a herbicidal compound.[1]

Table-1: Growth inhibitory activity of mimosine on growth of indicator plants (%).

Plant species	Concentration (mg/L)						
	1	10	25	50	100	1000	LSD (0.05)
			Radicle				
Brassica rapa	–10.4d	2.7b	89.4a	91.3a	94.8a	96.0a	9.1
Phaseolus vulgaris	–2.2c	41.0a	37.3c	46.3d	72.4b	83.7b	7.2
Bidens pilosa	–21.7e	2.4b	44.1c	38.0d	45.2d	62.0cd	11.4
Mimosa pudica	–34.0f	–17.8c	–6.3g	17.3e	40.1d	53.9d	12.3
Lolium multiflorum	1.4c	–24.1d	36.5c	74.6b	89.5a	97.8a	13.1
Leucaena leucocephala	7.9b	–78.4f	–7.4g	1.4f	2.4g	56.4cd	12.7
			Hypocotyl				
Brassica rapa	–3.3c	–2.9bc	71.2b	83.8a	92.8a	98.0a	10.5
Phaseolus vulgaris	9.8b	32.4a	27.9d	16.4e	44.2d	63.9c	7.7
Bidens pilosa	–13.1d	9.4b	44.1c	24.1e	40.6d	51.6d	6.9
Mimosa pudica	–28.3e	–12.0c	17.6e	60.2c	60.2c	61.3cd	8.2
Lolium multiflorum	16.4a	–33.0d	8.1f	24.0e	31.1e	64.7c	6.4
Leucaena leucocephala	–3.3c	–56.4e	–1.2g	2.8f	11.5f	61.1cd	5.9
LSD (0.05)	4.9	9.3	7.8	8.6	8.7	8.9	4.9

2. MATERIALS AND METHODOLOGY

The material and mythology follows to extracts the mimosine from the Leucaena-leucocephala de wit leaves is as follows. [4]

2.1 Raw Material Required

- Leucaena-leucocephala de wit leaves
- Distilled Water
- Ethanol
- Hydrochloric Acid
- Ortho-phosphoric

2.2 Equipment Required

- Glass Kettle 3 litre Capacity
- Pulveriser
- Sieve 20 Mesh
- 1000 ml size stoppered flask – Qty 01 Nos
- 500 ml size stoppered flask – Qty 02 Nos
- Constant Temperature Water Bath
- Buckler Filter
- pH meter
- Vacuum Pump
- HPLC

2.3 Methods of Extraction

1.Fresh leucaena leaves are collected from forest side. 1 kg of healthy leaves of Leucaena-leucocephala washed with plenty of fresh water to remove the dust adehered on the leaves, and then allowed to dry at room temperature for two days, as shown in fig-3.

Fig -3: Drying of Leucaena-leucocephala de wit leaves.

2. The dried leaves then crushed by hand and bark are separated through the screen of size 20 mesh. Then it is grind with pulveriser in powder form as shown in fig-4.

Fig -4: Leucaena leaves grinding.

3. A 750 ml distilled water is taken in the 1000 ml beaker to carry water extraction as shown in fig-5.

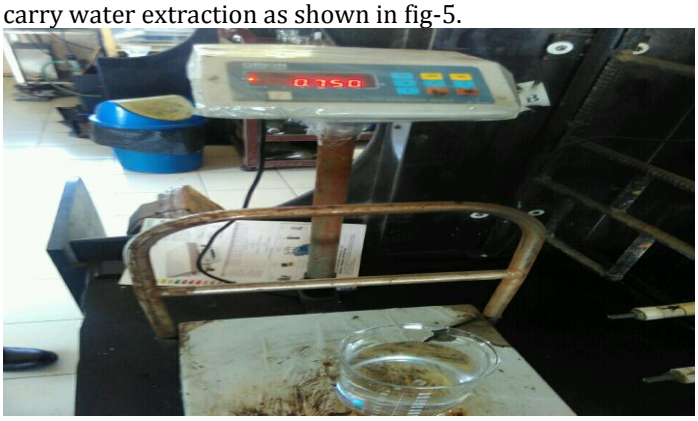

Fig -5: 750 gram distilled water

4. Then 150 gram leaves powder is taken for extraction, as shown in fig-6.

Fig-6: 150 gram, leucaena leaves powder

5. 750 gram distilled water and 150 grams of power is charged sequentially to 3 litre glass kettle. The kettle is placed in to constant temperature water bath to maintain the extraction mass temperature at 59 to 60 OC for 16-17 hours. The experimental set up is consist of reaction kettle placed in constant temperature water bath, equipped with glass condenser to minimise the vapour loss during extraction at 59 to 60 OC temperature by applying cooling water the condenser as shown in fig-7.

Fig-7: Water Extraction Kettle

6. After complete extraction, extraction mass is filter through buchner filter under vacuum and filtrate is collected in another 1000 ml size flask as shown in fig-8.

Fig-8: Cake and filtrate separated from extracted mass

7. Then the filtrate mass received from previous stage is added with 350 gram ethanol and allow for mimosine extraction for 24 hrs, as shown in fig9. During 24 hrs the solid form the extraction mass is settled at bottom and clear mass continuing ethanol and water solution is remain clear at top as supernatant as per fig-9.

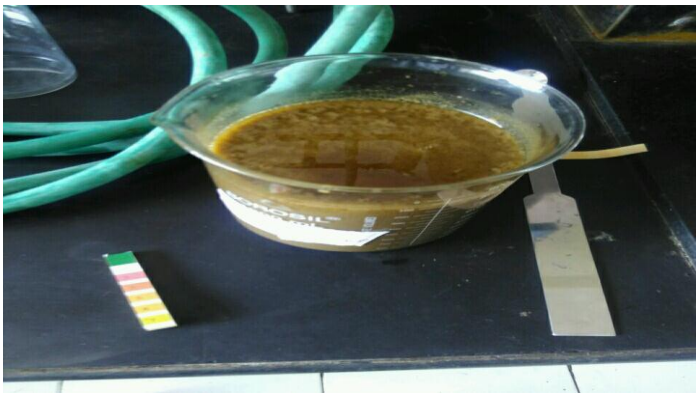

Fig-9: Extraction mass containing ethanol

8. After complete extraction the pH of extraction mass is adjusted by using HCL and pH is maintained at 5 by using pH meter.

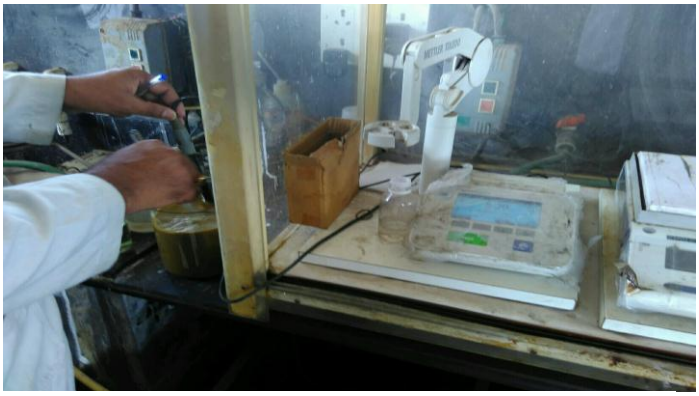

Fig-10: pH adjustment by pH meter

9. After pH adjustment the extraction mass is further filter through buchner filter under vacuum, and filtrate is collected 1000 ml size flask.

Fig-11: Vacuum filtration of ethanol extracted mass

10. The filtrate received from previous step is allowed to concentrate at 20 - 30 0C under vacuum, till the final material remains at volume of 20 grams only. This is done in distillation setup at lab scale as shown in fig-12.

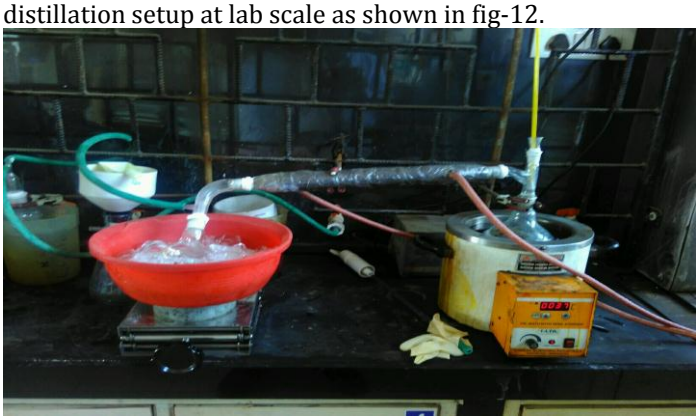

Fig-12: Concentration of mimosine supernatant by using vacuum pump

11. After completion of concentration the dark viscous material is remain in the re-boiler of distillation setup is collected in the 100 ml beaker and allowed to cool at room temperature. Further this mass is analysed to know the content of mimosine by using HPLC method.

Fig-13: Concentrate mass of containing mimosine.

2.4 Method of Analysis

Paper and thin layer chromatography were used to identify mimosine, however, mimosine content could not be quantified. Gas-liquid chromatography, liquid chromatography, and reversed-phase ion-pair high-performance liquid-chromatography were also applied for mimosine determination. However, these methods require elaborate preparation of samples, but with no appreciable improvement in the range of sensitivity.

Other methods were the coupling of mimosine with p-nitroaniline or mimosine with N-1(naphthyl)ethylenediamine (NEDA) forming a pinkcolored azodye with an absorbance of 540 nm, and the use of indirect spectrophotometricity which is based on its reaction with diazotized sulfanilamide. These methods were reported to increase the sensitive estimation of mimosine. A useful HPLC system to determine mimosine and DHP contents that influenced Rhizobium isolates was reported by Soedarjo et al. They applied a C18 HPLC column, UV detection at 280 nm, a solvent system of 0.2% orthophosphoric acid to detect mimosine and DHP at 2.7 and 4.8 min, respectively. [3]

3. RESULTS AND DISCUSSIONS

Mimosine extracted at lab scale is further analyzed by using HPLC method. A useful HPLC system having 880-PU pump and column Fine pak Sil C18 of Nihonbunko company is used to determine mimosine and DHP contents by using a solvent system of 0.2% ortho-phosphoric acid at wavelength of 280 nm, the peaks of mimosine and DHP were detected at retention time of 2.5 min and 7.4 min, respectively.

HPLC analysis report of mimosine extracted by using water and ethanol, shown in chart no: 1, shows the retention time of 2.595 min and 7.308 min respectively

Other impurities at retention time of 2.794 min, 3.724 min, 4.213 min, 4.213 min, 4.915 min, 8.223 min, 10.026 min, 11.462 min, 12.356 min, 13.806 min, and 29.662 min are unknown.

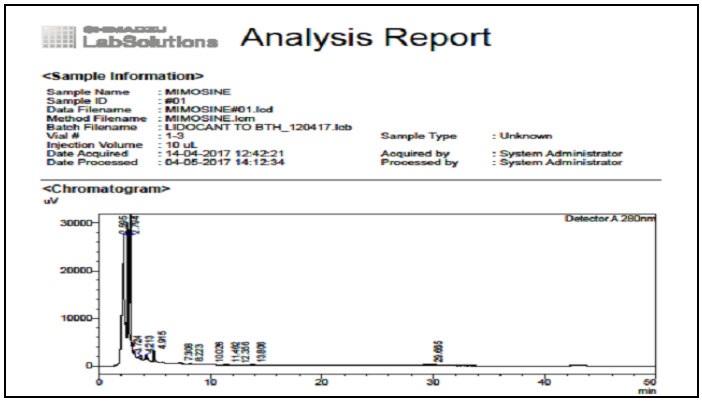

Chart -1: HPLC Analysis Report for Mimosine Extract

Table -1: HPLC Analysis Peak Table

<Peak Table>
Detector A 280nm

Peak#	Ret. Time	Area	Height	Area%	Name
1	2.695	119287	23612	12.572	MIMOSINE
2	2.794	757184	139936	79.804	UNKNOWN
3	3.724	7351	977	0.775	UNKNOWN
4	4.213	6761	980	0.713	UNKNOWN
5	4.915	19873	2272	2.095	UNKNOWN
6	7.308	5016	304	0.529	DHP
7	8.223	2037	116	0.215	UNKNOWN
8	10.026	968	55	0.102	UNKNOWN
9	11.462	2660	116	0.280	UNKNOWN
10	12.356	971	46	0.102	UNKNOWN
11	13.806	4774	169	0.503	UNKNOWN
12	29.665	21918	300	2.310	UNKNOWN
Total		948800	168883	100.000	

4. CONCLUSIONS

Momosine can be extracted by using different sources of legume tree, like mimosa pudica, leucaena-leucocephala. Mimosine can be extracted easily from leucaena-leucocephala leave by using water and ethanol as solvent. Extracted mimosine is determined by using HPLC method. Mimosine exhibited selective influence against the germination and growth of certain indicator plants including BidenspilosaL, Mimosa pudica, Brassica rapa, Loliummultiflorum, and Phaseoulus vulgaris.

REFERENCES

[1] T.D. Xuan, A.A. Elzaawely, F. Deba, M. Fukuta, S. Tawata. ''Mimosine in Leucaena as a potent bio-herbicide.'' Agronomy for Sustainable Development, Springer Verlag/EDP Sciences/INRA, 2006, 26 (2), pp.89-97. <hal-00886327>

[2] www.nabard.org/english/forestry_subabul.aspx

[3] Tran Dang Xuan, ShinkichiTawata and Tran Dang Khanh, '' Herbicidal Activity of Mimosine and Its Derivatives'' Herbicide – Advance in research, edited by Andrew J. Price and Jessica A Kelton, Publisher In Tech, May 2013. Chapter 15.

[4] https://patents.google.com/patent/CN103242224A/en?q=method&q=extracting&q=purifying&q=leucaena+leucocephala&q=leaf&q=mimosine

BIOGRAPHIES

M.Tech (II Year), Chemical Engg. College of Engineering & Technology, Akola (M.S), India

PHD, Professor, Chemical Engg. College of Engineering & Technology, Akola (M.S), India

PHD, HOD, Chemical Engineering Dept., College of Engineering & Technology, Akola (M.S), India