

## Preparation and Estimation of Metal Complexes of Glabrin from *Pongamia glabra* Linn. Seeds

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### INTRODUCTION

Glabrin (2-Piperidine, carboxylic acid, 4, 5, dihydroxy, -1-methyl) was isolated from *Pongamia glabra* seeds and metal complexes were prepared by refluxing with acetates of copper, zinc and mercury, estimation was carried out by simple idometric method for copper complex and E.D.T.A. method for zinc and mercury complexes of glabrin. Results of present study reveal that the metal complexes show calculated and theoretical yield as for copper 15.12/15.36% for zinc 15.36/15.74% and for mercury 36.30%/36.37% of metals.

*Pongamia glabra* Linn<sup>1</sup>, is commonly known as Karanja belongs to family Leguminosae. It is distributed throughout India especially near the banks of rivers and streams. Its seed oil has been used as folk remedies to cure many ailments like eye, skin and vaginal diseases<sup>2</sup>. It is also used in tumors, urinary discharges, as anthelmintic, antileprotic, antitubercular, pesticidal, antifungal and in deodorant soap preparations<sup>2</sup>. A number of phytoconstituents<sup>3,4</sup> have been isolated from the seeds i.e. karanjin, kanjone, Pongapin, pongaglabone, pongamol, isolanchocarpin, pongachromene, kanugin, glabrin and glabrachromene<sup>5,6</sup>. In present work, glabrin was isolated and metal complexes were prepared and these complexes were

estimated by simple assay methods for theoretical and percentage yield of metals in complexes of glabrin.

### MATERIALS AND METHODS

Seeds of *Pongamia glabra* were purchased from the local market and identity was confirmed by the Department of Botany, Dr. Hari Singh Gour Vishwavidyalaya, Sagar (M.P.). Seed kernels (2 kg) were reduced to moderately coarse powder and were extracted with petroleum ether (60-80<sup>0</sup>C) in a soxhlet extractor. The defatted marc was air dried and weighed (1.2 kg).

This defatted material was extracted with methanol in a soxhlet extractor for 60 hours. Thus obtained methanolic extract was kept a side for a few days for deposition of crystalline solid that began to separate. It was then filtered and washed with light petroleum. This was the mixture of karanjin and glabrin and components were separated easily because of their differences in solubility in alcohol. The one that went easily into alcohol was karanjin. The other left, was dissolved in the minimum amount of water (10 cc) and then filtered so as to remove all the adhering impurities. To the clear solution alcohol was added in order to precipitate the substance and then an excess

of water was added to the aqueous alcoholic solution. Glabrin separated as shining, colourless, crystalline solid (Yield 0.780 gm). The purity of glabrin was confirmed by melting point determination, spectroscopy and by Co-TLC.

### Preparation of Metal Complexes

Zinc, copper and mercury compounds of glabrin were prepared by refluxing metal acetates with aqueous solution of glabrin in 1:2 ratios of their molecular weights. Formation of metal complexes was confirmed by negative characteristic tests for  $\alpha$ -amino acid, which is positive for glabrin and were found to be negative in case of complexes. Structures of complexes were further confirmed by chemical assay.

### Estimation of Copper

The iodometric method<sup>7</sup> has been used to estimate the copper in the prepared complex. Accurately weighted 0.20 g of sample was taken in a small beaker and treated with water and 0.20 mg of sodium hydroxide and boiled till the precipitation was complete. The precipitate was filtered and washed thoroughly with hot water and dissolved in hot solution of nitric acid (1 part nitric acid and 4 parts water). This was then cooled and a little amount of water was added and boiled to drive off the excess of nitric acid. Again some water and ammonia added till it become distinctly alkaline. It was boiled for a few minutes to expel out the excess of ammonia. Complete removal of ammonia was shown by change in colour and partial precipitation. To this 0.30ml of acetic acid was added and boiled. Now it was cooled and 10ml of 3% potassium iodate was added and titrated with N/100 sodium thiosulphate using mucilage of starch as indicator. The whole procedure was repeated thrice.

1ml of N/100  $\text{Na}_2\text{S}_2\text{O}_3$  = 0.0006354 g of Cu

### Normality of Sodium Thiosulphate

Approximately N/100 solution of sodium thiosulphate was prepared and standardized against potassium dichromate (AR).

Normality of  $\text{Na}_2\text{S}_2\text{O}_3$  = 0.0099

$$\text{Percentage of Cu} = \frac{\text{Vol. of } \text{Na}_2\text{S}_2\text{O}_3 \times \text{Normality} \times 100 \times 0.0006354}{\text{Wt. in grams} \times 0.01}$$

### Estimation of Zinc

Estimation of zinc in metal complexes of glabrin was performed using EDTA method<sup>8</sup>.

#### Reagents:

#### 1. Erichrome Black T Indicator

Solution was prepared by dissolving 0.2 g of dyestuff in 15ml of triethanolamine and 5ml of absolute ethanol.

#### 2. E.D.T.A. Solution 0.01M

1.86 g of A.R. disodium dihydrogen ethylene diamine tetramine dihydrate was dissolved in water and was diluted to 500ml in a volumetric flask with redistilled water.

#### 3. Calcium ion solution

About 0.100 g accurately weighted calcium carbonate A.R. was transferred to a 100 ml volumetric flask with the help of little water, to it hydrochloric acid (6ml) was added drop by drop till effervescence ceases and the solution is clear. Sodium hydroxide is added to make it neutral and finally the volume

was made up to 100ml and 10 ml of this solution was diluted to 100ml.

#### 4. Ammonium chloride buffer (pH10)

Concentrated ammonia solution (14.2 ml.) was added to 1.72 g. of ammonium chloride and diluted up to 25ml with distilled water.

#### 5. Zinc ion solution

Compound to be estimated for zinc was accurately weighed and treated with hydrochloric acid. The resulting solution was nearly neutralized with sodium hydroxide solution and diluted with distilled water.

### Standardization of E.D.T.A. Solution

25ml of calcium solution was pipetted out in a 250ml conical flask and 1ml of ammonia-ammonium chloride buffer was added. It was titrated with E.D.T.A. using 2-3 drops of Erio-T indicator until colour changes from wine red to clear blue.

### Estimation of Zinc

To the above-diluted zinc ion solution 1.0ml of ammonia-ammonium chloride buffer and 2-3 drops of indicator were added. This was titrated with E.D.T.A. solution until the colour changed from wine red to blue.

1 ml. of 0.01 M.E.D.T.A. solution = 0.6538mg zinc.

Molarity of E.D.T.A. solution = 0.01027

$$\text{Percentage of Zinc} = \frac{\text{Volume of E.D.T.A.} \times \text{Molarity of E.D.T.A.} \times 100 \times 0.6538}{\text{Wt. in mg.} \times 0.01}$$

### Estimation of Mercury

Compound to be estimated for mercury was accurately weighed and treated with hydrochloric acid. The resulting solution was neutralized with M NaOH and diluted with distilled water. To the above diluted mercury ion solution 1ml. of ammonia-ammonium chloride buffer with standardized E.D.T.A. solution until the colour changes from wine red to blue.

1ml of 0.01 M, E.D.T.A solution. = 2.0059 mg Hg.

$$\text{Percentage of mercury} = \frac{\text{Vol. of E.D.T.A.} \times \text{Molarity of E.D.T.A.} \times 100 \times 2.005}{\text{Wt. of compound in mg.} \times 0.01}$$

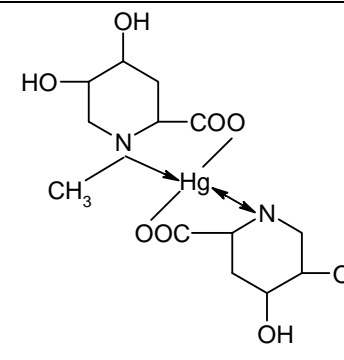
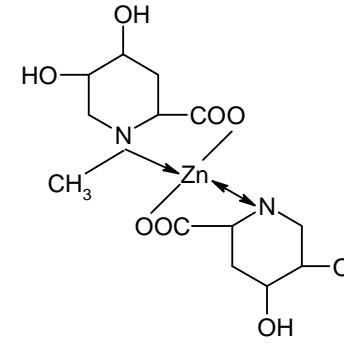
### RESULTS AND DISCUSSION

Glabrin (2-Piperidine carboxylic acid 4-5 dihydroxy -1 methyl) was isolated from the defatted methanolic extract of the seeds of *Pongamia glabra*. Purity of glabrin (Figure - 1) was confirmed by Co-TLC, spectrophotometry and melting point determination (228<sup>0</sup> C). Percent yield of compound was calculated as 0.039% w/w of the seeds. Copper, Zinc and Mercury complexes were prepared by refluxing metal acetates with aqueous solution of glabrin. Formation of metal complexes was confirmed by negative characteristic test for  $\alpha$ -amino acid, which is positive for glabrin and percent weight of metal complexes, which is in accordance with structure and theoretical ratio of molecular weight of the complexes. Estimation of metals in complexes was carried out by simple idometric method for copper complex and E.D.T.A. method for zinc and mercury complexes. Calculated percentage of metals in complexes coincided with theoretical percentage (Table.1) .

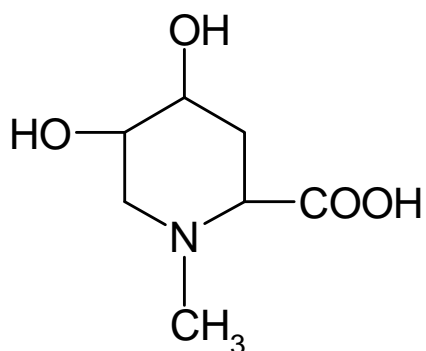
In the present investigation, isolation of glabrin, preparation of metal complexes and estimation of its metal complexes indicate that these methods are simple and precise. Glabrin and its metal complexes reported to have significant anti-microbial activity<sup>9</sup>,

These finding suggests towards further opportunity to explore some new pharmacological activities of complexes.

**Table.1 : Estimation of Mercury, Zinc and Copper**

S. No .	Wt. of compound (g)	Volume used (ml)	Calculated percentage	Mol. formula of complex	Structural formula of complex	Theoretical percentage of complex (%)
1.	0.02048	3.6 [E.D.T.A .]	36.32 (Hg)	$C_{14}H_{26}O_8N_2$ Hg		36.37
2.	0.02016	4.7 [E.D.T.A .]	15.43 (Zn)	$C_{14}H_{36}O_8N_2$ Zn		15.74

3.	0.02021	4.8 [Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ]	15.09 (Cu)	C <sub>14</sub> H <sub>26</sub> O <sub>8</sub> N <sub>2</sub> Cu		15.36
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**Galbrin**

## REFERENCES

1. Chopra, R.N., Nayar, S.L. and Chopra, I.C., "Glossary of Indian Medicinal Plants", C.S.I.R., New Delhi (1956), 318.
2. Kirtikar, K.R., Basu, B.D. "Indian Medicinal Plants", Lalit Mohan Basu, Allahabad, II-Ed., Vol. I (1976), 31.
3. Charak Samhita", Chikitsa Sansthanam, Barakhaba, Varanasi (1972), 736.
4. Aneja, R. and Khanna, N.R., *Ind. J. Pharm.* (1940), 83.
5. Parmar, B.S., Sahrawat, K.C. and Mukherjee, S.K., *J. Sci. Ind. Res.* (1976), 610.
6. Lakshmi, P., Srimannarayana, G. and Subbarao, N.V., *Ind. J. Chem.* (1974), 12(1),
7. Indian Pharmacopoeia, 1996, Vol II, 210.
8. Nayak, S.P. and Bringi, N.V., *Ind. J. Chem.* (1973), 11(11), 1189.
9. Chandel, H.S. Nayak, S. and Khare, R.L., *Planta Indica*, (2005), 1 (2), 27.