

# CHEMISTRY SECTION

## A STUDY OF THE ALKALOIDAL CONTENT OF *ATROPA BELLADONNA* L. GROWN IN EGYPT

A. HIFNY SABER, S.I. BALBAA AND M. S. KARAWYA

*Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, United Arab Republic*

Although a vast number of papers have been published dealing with *Atropa belladonna* L., the plant is still attracting further investigations.

With regard to the total alkaloidal content of *Atropa belladonna* L., great differences have been reported in the literature. Rowson<sup>1</sup> reported that good samples of dried belladonna herb contain 0.25 to 0.9% and the dried root 0.3 to 1.0% of total alkaloids.

Daleff et al.<sup>2</sup> reported that the highest alkaloidal content was found in the roots during the period of bud formation, and in the aerial parts the maximum was found during the same period and at the commencement of flowering. Atkinson and Melville<sup>3</sup> stated that as flowering commences, the alkaloidal content falls rapidly, then rises again and finally falls with the ripening of the fruit, and that the stem does not share in the second rise. With the flowering shoots and leaves, however, the second maximum is more marked than with the first.

Taie,<sup>4</sup> working on samples of leaves and roots of belladonna collected from various regions of Argentina, reported that they contain an average alkaloidal content of 0.45 to 0.65 calculated for 8% moisture, leaves and roots not differing much from each other. Zbigniew Kubiak<sup>5</sup> stated that the alkaloidal content changes in the stem during the vegetative period between 0.16 and 0.55%.

With regard to the nature of the alkaloids of *Atropa belladonna* L. plant, great variation in the findings published is also noticed. Phokas and Steinegger,<sup>6,7</sup> using partition chromatogra-

phy, found that belladonna root contained hyoscyamine as the chief alkaloid (approximately 90% of the total) together with apoatropine, scopolamine, belladonine and cuscohygrine. They also found that great inconsistencies in the occurrence of alkaloids were noticed in drugs obtained from varying places; notably a great content of cuscohygrine in drugs from Greece and Kashmir, in contrast with those from Switzerland, Bulgaria and Yugoslavia.

Reinouts Van Haga<sup>8</sup> reported that cuscohygrine is a normal constituent alkaloid of *Atropa belladonna* L.

Rowson<sup>1</sup> mentioned that *l*-hyoscyamine is the main alkaloid in *Atropa belladonna* L. herb and root occurring with small amounts of *l*-hyoscyne and of optically inactive atropine.

Rowson<sup>9</sup> reported also that the hyoscyne content of the total alkaloidal mixture was found to be remarkably constant between 5 and 11%.

Evans and Partridge<sup>10</sup> found that the greatest change in the ratio of hyoscyamine usually occurs at times of greatest meristematic activity. Reinouts Van Haga<sup>11</sup> reported that extracts of the germinating seeds and of seedlings showed in paper chromatography an unidentified alkaloid, probably belladine, but hyoscyamine, scopolamine, apoatropine, belladonine and atropine were not found.

In February, 1958, the cultivation of *Atropa belladonna* was tried in the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, and it was found that the plants grew







shaken with successive portions of chloroform, the chloroform evaporated and left on a boiling water-bath for 45 minutes. The residue was then dissolved in as little chloroform as possible. The chloroform solution was dropped in the form of a narrow band 1 cm. wide on the start line of a sheet of Whatman filter paper No. 1, 19 cm. broad and the paper developed as mentioned before.

A longitudinal strip, 1 cm. wide, was cut from the middle of the chromatogram and sprayed with modified Dragendorff reagent and used as a pilot for locating the position of the band on the rest of the chromatogram. This strip was placed again in its original position with the other two cut parts of the chromatogram and the bands at the level of the located spot were marked. The two marked

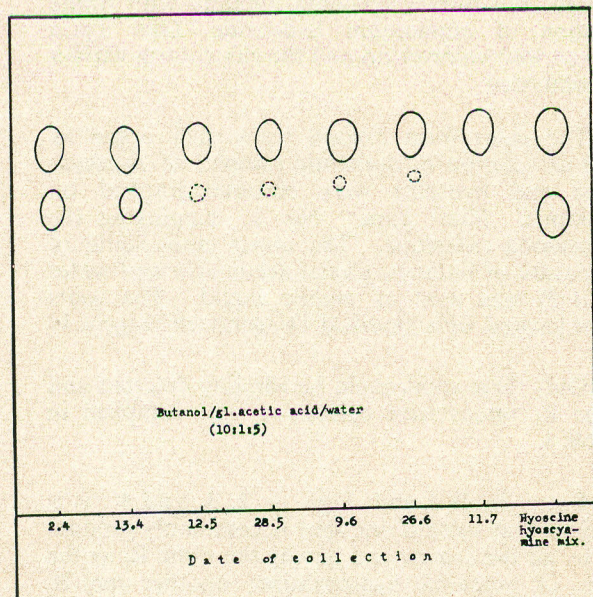


Fig. 1.—Chromatogram of alkaloidal extract of roots of *A. belladonna* at different stages of growth.

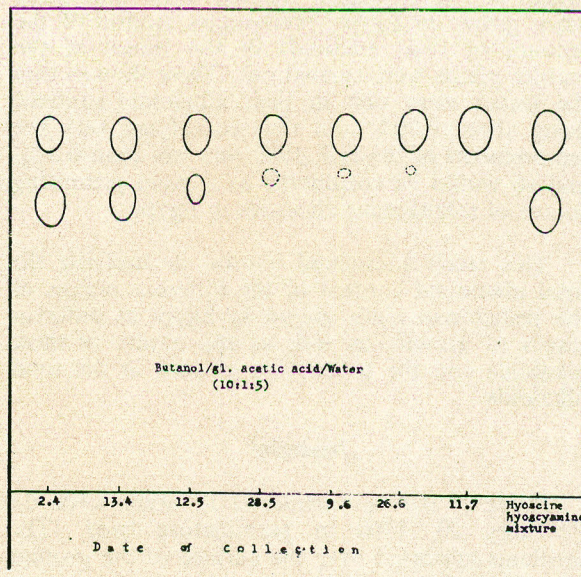


Fig. 2.—Chromatogram of alkaloidal extract of stems of *A. belladonna* at different stages of growth.

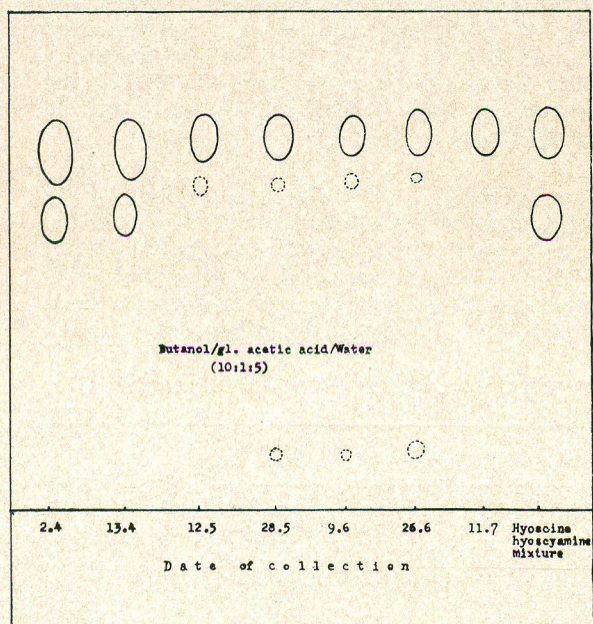


Fig. 3.—Chromatogram of alkaloidal extract of leaves of *A. belladonna* at different stages of growth.

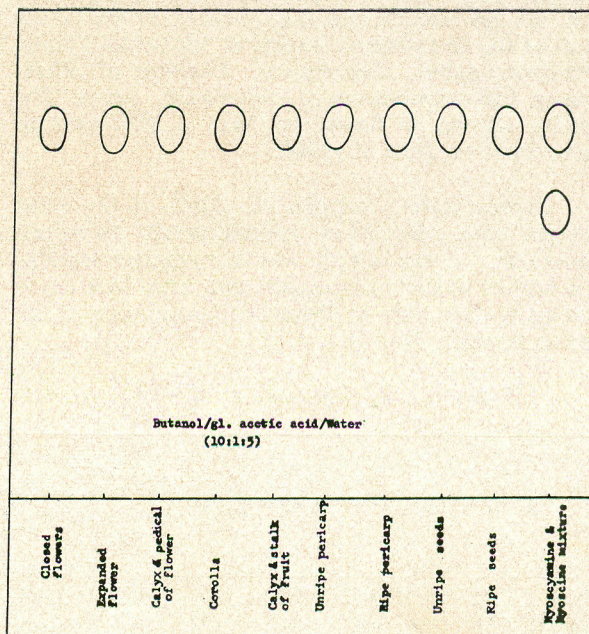


Fig. 4.—Chromatogram of alkaloidal extract of different parts of flowers and fruits of *A. belladonna*.



bands corresponding to spot A and spot B were then cut transversely across the chromatogram and the content in each band was eluted with alcohol. Auric chloride and picrate of the eluted substances were prepared and their m.ps. determined and found to be identical with those of hyoscyamine and hyoscine, respectively.

Spot C was rather too small that the substance producing it could not be separated and identified. Further work is now being undertaken for this purpose.

#### *Determination of the Total Alkaloidal Content in the Organs of Different Stages of Growth*

About 5 to 10 g. of the material, accurately weighed, was extracted with alcohol (70%), extract concentrated on a boiling water-bath, acidified with N/10 sulphuric acid, filtered into a separating funnel and washed with acid, till free from alkaloids. The acid solution was rendered alkaline with ammonium hydroxide solution and then the alkaloids completely extracted with successive portions of chloroform. The combined chloroform extracts were washed with 5 c.c. distilled water, chloroform evaporated and the residue heated on a boiling water-bath for 45 minutes adding 5 c.c. of neutral alcohol every 15 minutes.

The residue was then dissolved in 2 c.c. chloroform, 20 c.c. N/100 sulphuric acid added, the chloroform allowed to evaporate on a water-bath and the solution titrated against N/100 sodium hydroxide using methyl red as indicator. The results are compiled in Table 2 and represented by Figs: 5, 6, 7 and 8.

#### *Estimation of Hyoscyamine and Hyoscine by partition column chromatography*

The Evans and Partridge method<sup>13</sup> for the determination of hyoscyamine and hyoscine by buffered Kieselguhr as modified and improved by Flueck and Bettschart<sup>14</sup> was adopted. The method may be described briefly as follows :

The titrated liquid left from the determination of the total alkaloids of each organ of the plant at different stages of growth was rendered alkaline with ammonium hydroxide solution, alkaloids having been extracted with chloroform; chloroform was distilled off and the residue of alkaloids dissolved in 4 c.c. of a mixture of ether and chloroform 9:1. The alkaloidal solution was transferred on to a chromatographic column, 2 cm. diameter, containing 10 g. of Kieselguhr\* previously thoroughly mixed with 5 ml. of M/2 phosphate buffer (pH 7) and washed with 20 c.c.

ether-chloroform mixture. The column was then eluted with about 70 c.c. of the same ether-chloroform mixture or till exhaustion and the collected eluate containing hyoscine was evaporated. The residue was dissolved in 5 c.c. neutral alcohol; 20 c.c. N/100 sulphuric acid were added and titrated against N/100 sodium hydroxide using methyl red as indicator, the hyoscine content calculated

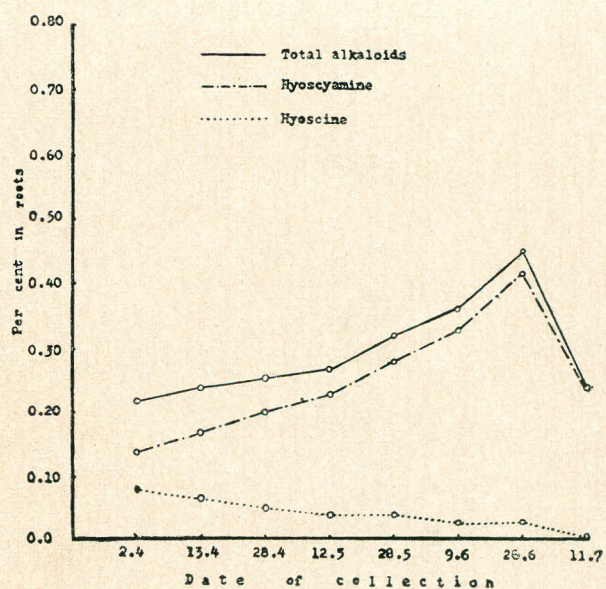


Fig. 5.—Percentage of total alkaloids, hyoscyamine and hyoscine in the roots of *A. belladonna* at different stages of growth.

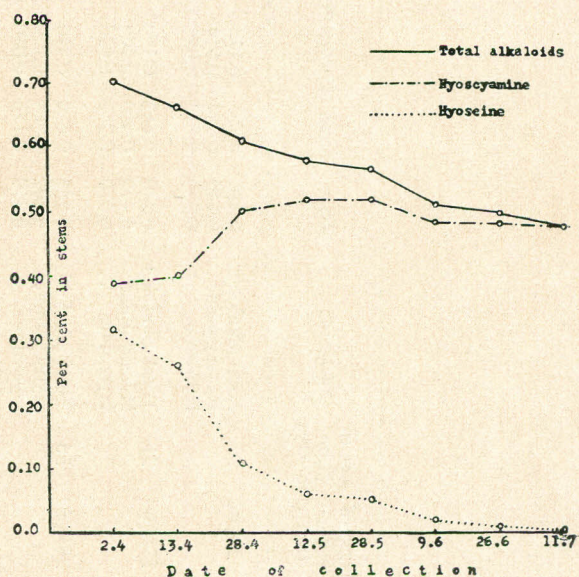


Fig. 6.—Percentage of total alkaloids, hyoscyamine and hyoscine in the stems of *A. belladonna*.

\*Column of more than 10 gm. of kieselguhr was used for amounts of alkaloids more than 0.02 gm.



TABLE 8.

Date of collection	Age of plant in days	Alkaloids	Root	Stem	Leaves	Petiole	Lamina	Flower		Calyx & pedicel of flower	Corolla of flower	Clayx & stalk of fruit	Pericarp		Seeds		
								Closed	Expanded				Unripe	Ripe	Unripe	Ripe	
2/4/60	10	Total alkaloids	0.220	0.701	0.160	—	—	—	—	—	—	—	—	—	—	—	—
		Hyoscyamine	0.140	0.384	0.070	—	—	—	—	—	—	—	—	—	—	—	—
		Hyoscine ..	0.080	0.317	0.090	—	—	—	—	—	—	—	—	—	—	—	—
13/4/60	21	Total alkaloids	0.240	0.662	0.190												
		Hyoscyamine	0.170	0.400	0.100												
		Hyoscine ..	0.070	0.262	0.090												
28/4/60	36	Total alkaloids	0.252	0.610	0.310												
		Hyoscyamine	0.202	0.500	0.230												
		Hyoscine ..	0.050	0.110	0.080												
12/5/60	50	Total alkaloids	0.268	0.580	0.320												
		Hyoscyamine	0.230	0.519	0.250												
		Hyoscine ..	0.038	0.061	0.070												
28/5/60	66	Total alkaloids	0.320	0.567	0.410	0.450	0.408	0.340	0.300	0.320	0.146	—					
		Hyoscyamine	0.280	0.521	0.360	0.370	0.399	0.340	0.300	0.320	0.146						
		Hyoscine ..	0.040	0.046	0.050	0.080	0.090	0.000	0.000	0.000	0.000						
9/6/60	78	Total alkaloids	0.360	0.508	0.550	0.581	0.502										
		Hyoscyamine	0.330	0.487	0.525	0.554	0.481										
		Hyoscine ..	0.030	0.021	0.025	0.027	0.021										
26/6/60	95	Total alkaloids	0.450	0.495	0.650	0.711	0.620						0.322		0.351		
		Hyoscyamine	0.420	0.486	0.639	0.697	0.602						0.322		0.355		
		Hyoscine ..	0.030	0.009	0.011	0.014	0.018						0.000		0.000		
11/7/60	110	Total alkaloids	0.240	0.475	0.720	0.770	0.700					0.312		0.150		0.460	
		Hyoscyamine	0.240	0.475	0.720	0.770	0.700					0.312		0.150		0.460	
		Hyoscine ..	0.000	0.000	0.000	0.000	0.000					0.000		0.000		0.000	



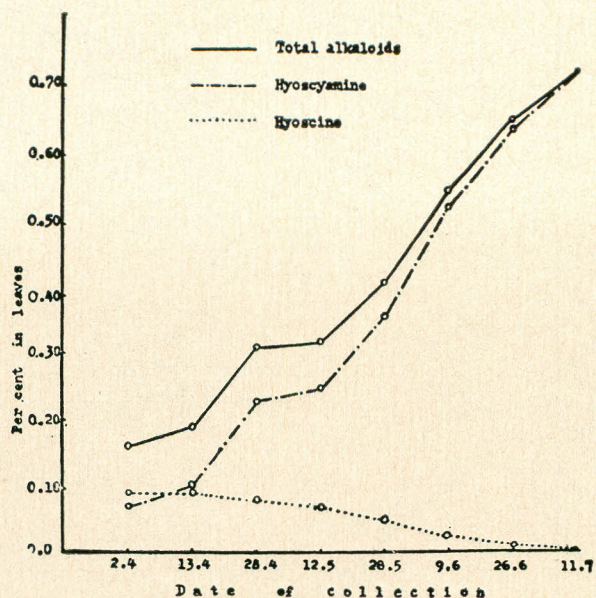


Fig. 7.—Percentage of total alkaloids, hyoscyamine and hyoscyne in the leaves of *A. belladonna* at different stages of growth.

as hyoscyamine being subtracted from the total alkaloid to give the hyoscyamine fraction. Results are compiled in Table 2 and represented by Figs. 5, 6 and 7.

From Table 2 and Figs. 5, 6 and 7 one may conclude the following :

#### I. Total Alkaloidal Content.

1. The percentage of the total alkaloids in the roots increases gradually with the growth of the plant and reaches its maximum when the plant is about three months old when it attains 0.45 per cent and then it decreases in the fruiting stage.

2. In the stem, the percentage of the total alkaloids is high in the young plant being 0.701% in the 10-day old seedlings and then it decreases gradually to 0.475% in the fruiting plant.

3. In the leaves, the alkaloidal content is low in the young plants; being 0.16% in the 10-day old seedlings, and then increases attaining 0.72% in the fruiting plant.

4. In the petiole and lamina of the leaf, the percentage of the alkaloid increases gradually during the growth of the plant and the petiole is richer in its alkaloidal content than the lamina; reaching 0.77% in the petiole and 0.70% in the lamina in the fruiting stage.

5. The closed flowers contain 0.34% of total alkaloids and the expanded flowers 0.39%.

6. The calyx with gynaecium and pedicel of the flower contain 0.32% of the total alkaloids but the corolla and stamens attached is poorer in its alkaloidal content, being 0.146%.

7. The calyx and stalk of the fruit contain 0.312% of total alkaloids.

8. The pericarp of the unripe fruit is richer in its alkaloidal content (0.322%) than the pericarp of the ripe fruit (0.150%).

9. The unripe seeds contain 0.351% of total alkaloids but on ripening, the alkaloidal content increases up to 0.46%.

#### II. The Hyoscyamine and Hyoscyne Content :

a. In the Plant: 1. In the roots of the young plants the hyoscyamine content is 0.14% and the hyoscyne content is 0.08% but during the growth of the plant, the proportion of hyoscyamine increases while that of hyoscyne decreases; the hyoscyamine being 0.42% and the hyoscyne 0.03% in the 95-day old plant.

In the fruiting plant, 110 days old, the root contains only hyoscyamine (0.24%) and is devoid of hyoscyne.

2. The percentage of hyoscyamine in the stem of the seedlings (10-day old) is 0.384% and the hyoscyne is 0.317% and during the growth of the plant the hyoscyamine increases while the hyoscyne decreases till the plant is about 95 days old when the hyoscyamine content is 0.486% and the hyoscyne content is 0.009%.

In the fruiting stage (110-day old plants) the stem is devoid of hyoscyne and contains only hyoscyamine (0.475%).

3. The hyoscyne content in the leaves of the 10-day old seedlings (0.09%) is more than the hyoscyamine content (0.07%), but the hyoscyne content decreases gradually till it becomes absent in the fruiting plant, while the hyoscyamine content increases gradually reaching up to 0.72% in the fruiting plant.

The hyoscyamine and hyoscyne content of both the petiole and the lamina behaves similarly as that in the leaves.

4. The closed and expanded flowers as well as the unripe and ripe fruits and seeds contain only hyoscyamine, although the chromatograms of the expanded flowers as well as of the calyx and stalk of the fruits showed traces of hyoscyne which could not be determined by column chromatography.



b. In the Total Alkaloids (Table 3 and Fig.8):—

With the exception of the leaves of the 10-day old plant, the hyoscyamine is the principal alkaloid in belladonna and its percentage in the total alkaloids increases as the plant grows older till it is 100% in the fruiting stage or in other words it is the only alkaloid present in fruiting belladonna plant. On the other hand, the hyoscine decreases till it is negligible and underterminable in the fruiting plant.

Spot C produced by the unidentified substance was noticed in the chromatograms, only in the case of roots of the 66, 78 and 95-day old plant, i.e. when the plant is flowering till the early fruiting stage but it is noticed neither in the case of roots in the other stages of growth of the plant nor in the case of the other organs in any stage of growth.

TABLE 3.—PERCENTAGE OF HYOSCYAMINE AND HYOSCINE IN TOTAL ALKALOIDS OF BELLADONNA.

Date of collection	Alkaloid	Percentage in			
		Root	Stem	Leaves	
2/4/60	Hyoscyamine	..	63.6	54.8	43.4
	Hyoscine	..	36.4	45.2	56.8
13/4/60	Hyoscyamine	..	70.8	60.4	52.6
	Hyoscine	..	29.2	39.6	47.4
28/4/60	Hyoscyamine	..	80.1	81.9	74.2
	Hyoscine	..	19.9	18.1	25.8
12/5/60	Hyoscyamine	..	85.8	89.5	78.1
	Hyoscine	..	14.2	10.5	21.9
28/5/60	Hyoscyamine	..	87.5	91.8	87.8
	Hyoscine	..	12.5	8.2	12.2
9/6/60	Hyoscyamine	..	91.6	95.8	95.4
	Hyoscine	..	8.4	4.2	4.6
26/6/60	Hyoscyamine	..	93.3	98.2	96.7
	Hyoscine	..	6.7	1.8	3.3
11/7/60	Hyoscyamine	..	100.0	100.0	100.0
	Hyoscine	..	0.0	0.0	0.0

### Summary

1. Belladonna grows well and robust in the Experiment Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, Cairo, U.A.R.

2. The alkaloidal content, being followed in the different organs during the growth of the plants; the highest being in the leaves (0.72%) of the fruiting plants.

3. Hyoscyamine and hyoscine were detected and determined in the plant by chromatographic methods.

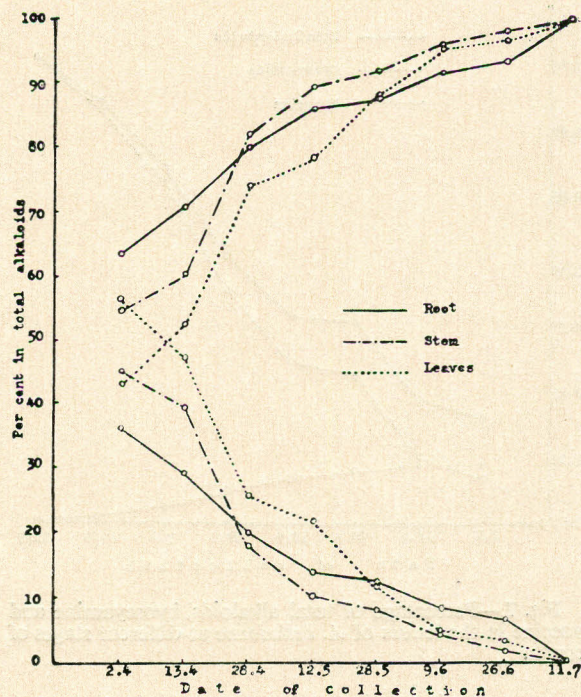


Fig. 8.—Percentage of hyoscyamine and hyoscine in total alkaloids of roots, stem and leaves of *A. belladonna* at different stages of growth.

4. Hyoscyamine is the principal alkaloid, being present in all organs in the different stages of growth but in variable amounts reaching up to 0.72% in the leaves.

5. Hyoscine was detectable in the leaves, stems and roots, decreasing during the growth of the plant till it disappears in the fruiting plant. It is also absent in the flowers, fruits and seeds.

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