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EFFECT OF HOST PLANT ON THE CHEMICAL COMPOSITION OF CUSCUTA REFLEXA ROXB

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Cuscuta reflexa Roxb. collected from *Zizyphus jujuba*, *Clerodendron inerme*, *Citrus medica* and *Accacia arabica*, has been investigated with a view to examining if its composition changes with the change in the host. Petroleum ether, diethyl ether, ethanol and water extractives of the parasite have been examined for their composition. No influence of the host plant has, so far, been found on the chemical built-up of this parasitic climber.

Introduction

Cuscuta reflexa Roxb. is a well-known leafless parasitic climber with long twining yellowish green stems and white sweet-scented flowers. It thrives on many trees and bushes, showing a marked preference for certain species, e.g. *Zizyphus vitex*.[1] Growing on various trees it is found throughout the country and has extensively been employed in Unani system of medicine for the treatment of a variety of ailments.[2] This parasite has been studied by various workers[3–8] and there are conflicting reports as regards its chemical composition. For example, Agrawal and Dutt[4] reported the presence of cuscutalin and cuscutin in the stems, neither of which could be isolated by Gopinath *et al.*[3] Instead, the later workers reported the presence of a flavonoid pigment and β-sitosterol. Similarly, Agrawal[5] investigated the pigments of the parasite and found that they contained amarbelin only. Working on the colouring matter again, Gopinath[3] has reported that instead of amarbelin, it is constituted of kaempferol.

From the foregoing reports it was clear that although *C. reflexa* has been studied from time to time, in fact, no systematic investigation had been carried out. This was essential as no two previously reported observations agreed with each other. However, Subramanian and Nair[8] have ascribed the parasite's compositional variations to the influence of the host plant. Their hypothesis is based on an analogous study by Srimathi and Sreenivassaya[9] on *Santalum album* Lin.

The present work was thus undertaken with a view to studying the composition of *C. reflexa* Roxb. grown on botanically different species of host plants, *viz.*, *Zizyphus jujuba*, *Clerodendron inerme*, *Citrus medica* and *Accacia arabica*. The parasite could be grown easily and could, therefore, be had free from any material of the host plant. The purity of the sample was, therefore, assured.

As a result of the present investigations it has, so far, been observed that the composition of *C. reflexa* Roxb. does not vary with the change of the host plant. Various extractives of the parasite growing on different hosts have been shown to be identical as a result of the chemical analyses. It is quite possible that the differences in the composition reported earlier might have risen due to the foreign impurities imported from the host plant.

Experimental

All melting points were determined on a Kofler block and are uncorrected. Organic extracts were dried over anhydrous sodium sulphate.

Petroleum Ether Extract.—Fresh stems of *C. reflexa* (1300 g) from the hosts were dried identically, in shade at room temperature, and extracted with petroleum ether (40–60°) in a soxhlet extractor. After removing the solvent, a waxy mass (3.65 g) was obtained that was chromatographed on alumina and eluted in succession with petroleum ether, diethyl ether and chloroform. Solvents were removed from the eluates at room temperature under reduced pressure and the residues obtained were weighed and purified by crystallisation. These observations are recorded in Table 1.

Petroleum ether soluble fraction proved to be a hydrocarbon (m.p. 61–2°) as it did not depress the m.p. of a synthetically prepared hydrocarbon from the Wurtz reaction of n-tridecyl bromide. The diethyl ether soluble fraction was also an hydrocarbon (m.p. 76–78°). The chloroform eluates were identified to be β-sitosterol by comparison with the IR spectrum of a standard sample (m.p. and mixed m.p. 139–140°). Its acetate also had an undepressed melting point with that of an authentic sample (m.p. 133°).

Diethyl Ether Extract.—*C. reflexa* Roxb. samples which had been freed of petroleum ether soluble components, were then subjected to Soxhlet extraction with diethyl ether. After removing the solvent the residue in each case was triturated with diethyl ether and the insoluble portion left behind

TABLE 1.—COLUMN CHROMATOGRAPHY OF PETROLEUM ETHER EXTRACT.

Host plant	Fraction eluted (in g) with				Melting point of eluates		
	Petroleum ether	Diethyl ether	Chloroform	Total weight	Petroleum ether	Diethyl ether	Chloroform
Zizyphus jujuba	1.4956	0.3402	0.1842	2.0200	62–63°	76–78°	139–140°
Clerodendrone inerme	1.4570	0.3602	0.1700	1.9872	62–63°	76–78°	139–140°
Citrus medica	1.4848	0.3340	0.1770	1.9958	62–63°	76–78°	139–140°
Accacia arahica	1.4724	0.3182	0.1680	1.9586	62–63°	76–78°	139–140°

TABLE 2.—SUGAR COMPOSITION OF *Cuscuta reflexa* ROXB. ALONGWITH THEIR *Rf* VALUES.

Sugar	*Rf*
Glucose	0.21
Fructose	0.29
Galactose	0.13
Rhamnose	0.35

was dissolved in methanol. The ether-soluble portion was a semisolid yellowish brown uncrystallizable sticky mass. The methanol-soluble portion on purification gave a crystalline compound (from ethanol–ether mixture) which decomposed without melting at 118°C and was not investigated any further.

Alcoholic Extract.—The samples having been freed of petroleum ether and diethyl ether, soluble components were then extracted with ethyl alcohol in an aspirator for 100 hr. The solid residue left behind after the removal of the solvent was triturated with a mixture of acetone: ethyl acetate (1:1) and then filtered. The insoluble portion was dissolved in ethanol. The acetone: ethyl acetate solution gave a compound m.p. 226°C. It reduced a warm Fehling solution. The ethanol-soluble compound melted at 275°C and gave a green colour with alcoholic ferric chloride. On the evidence of the previously reported work these compounds were concluded to be bergenin and kaempferol respectively.[6]

Aqueous Extract.—After the petroleum ether, diethyl ether and ethanol extractions the *C. reflexa* Roxb. samples were extracted in an aspirator with distilled water for 48 hr. These extracts gave positive tests for the presence of carbohydrates.

The sugar solutions were de-ionised[10] with redistilled pyridine at 100° for 10 min and deproteinised by barium hydroxide–zinc sulphate heptahydrate method.[11] Aliquots from the de-ionised and deproteinised sample were applied to Whatman No. 1 filter paper. The solvent used for the development was 1-butanol: acetic acid: water (40:10:22) and the indicator was *p*-anisidine hydrochloride prepared according to Pridham's method.[12] The chromatograms were treated at 130°C for 10 min for full colour development. Results thus obtained are shown in Table 2 and Fig. 1.

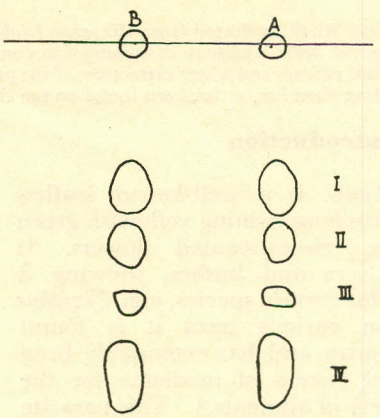

Fig. 1.—A, standards. B, sample; I, galactose; II, glucose; III, fructose; IV, rhamnose.

Amino Acid Composition

(i) *Nitrogen Content.*—The amount of nitrogen as determined by the method of Shah, Bhatty, Ashraf[13] was 1.45% on the basis of which total protein content came out to be 9% (1.45×6.25).

(ii) *Preparation of the Sample.*—(a) Acid Hydrolysis:[14] One g of the sample was hydrolysed under reflux with 10 ml 6N hydrochloric acid for 20 hr. The excess of the hydrochloric acid was removed by evaporation to dryness under reduced pressure at steam bath and the resulting mass consisting of amino acid hydrochlorides was placed in a desiccator over soda lime for 24 hr. The hydrolysate was, then, taken up in warm water, filtered, again evaporated to dryness and finally taken up in exactly 1 ml. 10% 2-propanol.

(b) Alkaline Hydrolysis:[15] One g of the sample was boiled with 10 ml barium hydroxide (14%) at 125°C under reflux for 20 hr. The barium ions were removed by precipitation with a slight excess of 1N sulphuric acid and the precipitate was thoroughly washed with hot water containing a drop of acetic acid. The filtrate was concentrated to a small volume under suction and then evaporated to dryness in a desiccator over anhydrous calcium chloride. The residue was taken up in 1 ml 10% 2-propanol.

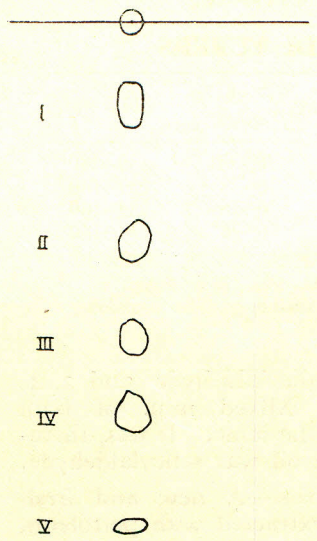

Fig. 2.—I, Aspartic acid; II, glutamic acid; III, serine; IV, threonine; V, lucine.

(iii) *Qualitative Paper Chromatography*.—The amino acids as obtained in (a) and (b) above were separately analysed by paper chromatography on a Whatman No. 1 filter paper which had previously been treated with 0.05% solution of potassium ferrocyanide (0.5g in 70 ml methanol and 40 ml water) and dried. This treatment was given to prevent bearding of spots and to reduce crinkling and warping of the paper on drying.[16] In the unidimensional procedure the solvent system was n-butanol–glacial acetic acid–water (4:1:5) whereas in the bidimensional phenol–water (80:30) and n-butanol–glacial acetic acid–water (4:1:5) were used. The chromatograms were developed by spraying a solution of ninhydrin (1% in acetone) and the spots were fixed by further spraying with a saturated aqueous solution of cupric nitrate (1 ml) in ethanol containing 10% nitric acid. The observed *Rf* values alongwith the possible amino acids were further confirmed by observing *Rf* values of a synthetic mixture of these amino acids.

(iv) *Quantitative Paper Chromatography*.—As the bidimensional procedure did not give any further separation of amino acids than the undimensional one, only the latter was used for their quantitative determination. The amino acids obtained by the acid hydrolysis of the sample were chromatographed in three sets of experiments by taking 20, 30, and 40 microlitres of the hydrolysate for each set respectively. The developed spots were eluted with a solution of copper sulphate (0.2 mg) in 20% aqueous ethanol (10 ml) separately. The resulting solutions were examined colorimetrically at 540 (Beckman DB), after they had stayed at room temperature for 2 hr. The percentage composition of the various amino acids determined from the percent transmission with the standard graphs for the amino acids, and the R*f* values obtained from Fig. 2 are recorded in Table 3.

TABLE 3.—AMINO ACID COMPOSITION OF *Cuscuta reflexa* ROXB. ALONGWITH THEIR *Rf* VALUES AND PERCENTAGES.

Amino Acid	*Rf*	Percentage
Aspartic acid	0.09	1.40
Glutamic acid	0.30	3.40
Serine	0.44	2.60
Threonine	0.55	0.90
Lucine	0.74	0.12

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References

1. R.N. Parker, *A Forest Flora for the Punjab with Hazara and Delhi* (Government Printing Press, Lahore, West Pakistan, 1956), third edition, p. 263.
2. R.N. Chopra *et al.*, *Indigenous Drugs of India* (U.N. Dhur and Sons, Private Ltd., Calcutta, 1958), second edition, p. 329.
3. K.W. Gopinath, R.C. Sharma and A.R. Kidwai, J. Sci. Ind. Res. (India), **21B,** 601 (1962).
4. R.R. Agrawal and S. Dutt, J. Indian Chem. Soc., **12,** 384 (1935).
5. R.R. Agrawal, J. Indian Chem. Soc., **13,** 531 (1936).
6. M.K. Jain and R. K. Mishra, Indian J. Chem., **1,** 499 (1963).
7. S.S. Subramanian, and A.G.R. Nair, Indian J. Chem., **1,** 501 (1963).
8. S.S. Subramanian and A.G.R. Nair, Indian J. Chem., **2,** 81 (1964).
9. R.A. Srimathi and M. Sreenivassaya, Current Sci. India, **32,** 11 (1963).
10. F.H. Malpress and A.B. Morrison, Nature, **164,** 963 (1949).
11. M. Somogyi, J. Biol. Chem., **160,** 61 (1945).
12. J.B. Pridham, Anal. Chem., **28,** 1967-68 (1956).
13. M. Ashraf, M.K. Bhatty and R.A. Shah, Pakistan J. Sci. Res., **12,** 103 (1960).
14. R. J. Block and D. Bolling, *Amino Acid Composition of Proteins and Foods* (C.C. Thomas, Springfield, Illinois, 1951), p. 576.
15. R. J. Block, E.L. Durram and Gunter Zweig, *A Mannual of Paper Chromatography and Paper Electrophoresis* (Academic Press New York, 1958), second edition, p. 119.
16. J. Trazaska and G.N. Kowakabany, J. Chromatog., **26,** 141-150 (1967).