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STUDIES ON THE STEM EXUDATE OF *AZADIRACHTA INDICA* LINN.

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THE stem exudate of *Azadirachta indica* Linn. (Neem), a rare exudation of stem of older trees, has been reported to be a nutritive and an alternative tonic[1,2] and found useful in the treatment of atonic dyspepsia, general debility, chronic leprosy and skin diseases[1]. The present work was undertaken to study the chemical composition of the exudate and to ascertain the presence if any of active medicinal principle in it.

The neem exudate, collected from an old tree situated at Dasna, Ghaziabad had a b.p. 97°, $[\alpha]_D^{32} + 46.87°$, pH of 3.5, sp. gr. of 0.997, moisture content of 92% and an ash content of 1.6% of the dry sap.

Free amino acids of concentrated exudate were determined by one-dimensional paper chromatography in *n*-BuOH-AcOH-H_2O (4:1:1) solvent system. The chromatograms, loaded with 25 μl of the extract and reference samples, were developed by spraying with ninhydrin and heating at 100°C for 5 min. The individual amino acids were eluted separately with 50% aqueous acetone and their optical densities were measured at 560 nm. In all, twelve amino acids were present in the exudate. The concentrations of γ-aminobutyric acid and glycine were the highest while arginine, glutamine, lysine and threonine were low (table 1).

The crude protein content (N × 6.25) (3.57 g/100 ml) of the exudate was determined by micro Kjeldhal's method as recommended by AOAC[3]. The protein, separated by adding ethanol (95%) to the concentrated exudate (50 ml), was washed several times with ethanol to remove the unbound amino acids and then hydrolysed with 20 ml of 6 N HCl in a sealed tube at 110°C for 24 h. The acid was removed in a rotatory film evaporator. The hydrolysed material was subjected to paper chromatography for analysing the bound amino acids. In all 14 amino acids were detected among which alanine, glycine and tyrosine were present in high concentrations (table 1).

The sugars glucose, fructose, mannose and xylose were detected by applying concentrated exudate on a paper chromatogram and developing

Table 1 *Free and protein bound amino acids of Azadirachta indica Linn. stem exudate*

Amino acids	Free amino acids (mg/l)	Protein amino acids (mg/100 g)
Alanine	328	486
γ-aminobutyric acid	486	–
Arginine	90	196
Asparagine	136	–
Aspartic acid	280	118
Cysteine	164	212
Glutamic acid	–	316
Glutamine	116	328
Glycine	542	416
Lysine	124	346
Methionine	–	142
Norleucine	–	294
Norvaline	254	112
Ornithine	–	248
Proline	266	–
Threonine	120	264
Tyrosine	–	432

the same in descending manner in *n*-BuOH-HOAc-H_2O (4:1:5, top layer), *n*-BuOH-EtOH-H_2O (4:1:2) and phenol saturated with water. Sucrose was isolated from silica gel column using $CHCl_3$-EtOH (1:1).

Total acid concentrations (9.184 g/lit) of the exudate and that of dried sap (5.60 g/l) were determined by standard sodium hydroxide. Citric, malonic, succinic and fumaric acids in the dried exudate were separated one-dimensionally on a paper chromatogram with *n*-BuOH-HCOOH-H_2O (4:1:5). The dried paper was sprayed with bromothymol blue to get yellow spots. Acetic acid was identified in the distillate of the sap.

The exudate was extracted with chloroform to separate steroids and limonoids. The extract was concentrated, subjected to column chromatography over silica gel after the formation of slurry and the column was run with benzene, chloroform and methanol in the order of polarity. Fractions (1–5) eluted with benzene gave β-sitosterol, m.p. and m.m.p. 140–141°, $[\alpha]_D^{32} - 36°$ (conc. 0.4 g in $CHCl_3$). Acetylation with acetic anhydride and pyridine gave an acetate, m.p. 130–132°.

Further elution of the column with benzene (6–9 fractions) gave 24-methylenecycloartanol, m.p. 120–121, $[\alpha]_D^{32} + 45°$ (conc. 0.3 g in $CHCl_3$), IR (KBr) 3400, 1629, 1010, 890 cm^{-1}.

Fractions (10–12) eluted with benzene-chloroform (9:1) yielded nimbin which on crystallization from methanol melted at 203–205°, $[\alpha]_D^{32} + 168$ (conc. 1 g in $CHCl_3$) (lit.[4] m.p. 201–204°, $[\alpha]_D + 170°$).

Fractions (13–15) eluted with benzene-chloroform (4:1) on repeated chromatography on silica gel gave azadirone in gummy form, $[\alpha]_D^{32} + 23°$ (conc. 0.55 g in $CHCl_3$) (lit.[5] $[\alpha]_D + 26°$), λ_{max}^{MeOH} 225 nm (9,980), IR (KBr) 1745, 1685, 1240, 885 cm^{-1}.

The fractions (16–20) collected from benzene-chloroform (1:1) on crystallization from methanol gave gedunin, m.p. 216–217°, $[\alpha]_D^{32} - 40°$ (conc. 1 g in $CHCl_3$), (lit[5]. m.p. 218°, $[\alpha]_D - 44°$), λ_{max}^{MeOH} 225 nm (9200), IR (KBr) 1745, 1675, 885 cm^{-1}.

β-Sitosterol and 24-methylenecycloartanol were the only two steroids obtained from the neem exudate. Earlier, these steroids were isolated from the leaves and heartwood of this plant[6,7]. The limonoids nimbin, azadirone and gedunin might be responsible for the medical properties of the sap. TLC of the chloroform extract indicated that at least three other limonoids might be present in trace amounts which could not be separated. The tonic properties of the sap might be due to the presence of amino acids, proteins, carbohydrates and salts in sufficient amounts.

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LOSS OF TOXIGENICITY OF *ASPERGILLUS FLAVUS* STRAINS DURING SUBCULTURING – A GENETIC INTERPRETATION

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THE loss of toxigenic potentials of fungal strains on repeated subculture has been reported[1]. The present authors examined the toxigenic potentials of *Aspergillus flavus* strains which elaborate aflatoxins (a well-known carcinogen and mutagen). Four strains of *A. flavus* [MZ from maize seeds with 4600 ppb potential; W-1 from wheat sample-1 with 2800 ppb potential; W-2 from wheat sample-2 with 1800 ppb potential and MC from mustard cake with 920 ppb potential] were isolated and grown on SMKY liquid medium[2] at 28 ± 2°C. After seven days the medium was filtered and extracted with chloroform. Qualitative and quantitative estimations[3,4] of aflatoxin were carried out. The subculturing was repeated seven times at regular intervals of seven days and the amount of aflatoxin produced at the end of each incubation period (i.e. 7–49 days) was estimated.

The results showed gradual decline in the toxin production. This decrease was almost rectilinear and