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 and found useful in the treatment of atonic dyspepsia, general debility, chronic leprosy and skin diseases. 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°C, [α]D +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-H2O (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 aceton 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. 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>
<thead>
<tr>
<th>Amino acids</th>
<th>Free amino acids (mg/l)</th>
<th>Protein amino acids (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>328</td>
<td>486</td>
</tr>
<tr>
<td>γ-aminobutyric acid</td>
<td>486</td>
<td>–</td>
</tr>
<tr>
<td>Arginine</td>
<td>90</td>
<td>196</td>
</tr>
<tr>
<td>Asparagine</td>
<td>136</td>
<td>–</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>280</td>
<td>118</td>
</tr>
<tr>
<td>Cysteine</td>
<td>164</td>
<td>212</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>–</td>
<td>316</td>
</tr>
<tr>
<td>Glutamine</td>
<td>116</td>
<td>328</td>
</tr>
<tr>
<td>Glycine</td>
<td>542</td>
<td>416</td>
</tr>
<tr>
<td>Lysine</td>
<td>124</td>
<td>346</td>
</tr>
<tr>
<td>Methionine</td>
<td>–</td>
<td>142</td>
</tr>
<tr>
<td>Norleucine</td>
<td>–</td>
<td>294</td>
</tr>
<tr>
<td>Norvaline</td>
<td>254</td>
<td>112</td>
</tr>
<tr>
<td>Ornithine</td>
<td>–</td>
<td>248</td>
</tr>
<tr>
<td>Proline</td>
<td>266</td>
<td>–</td>
</tr>
<tr>
<td>Threonine</td>
<td>120</td>
<td>264</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>–</td>
<td>432</td>
</tr>
</tbody>
</table>
the same in descending manner in n-BuOH-HOAc-
H₂O (4:1:5, top layer), n-BuOH-EtOH-H₂O
(4:1:2) and phenol saturated with water. Sucrose
was isolated from silica gel column using CHCl₃-
EtOH (1:1).

Total acid concentrations (9.184 g/lit) of the
exudate and that of dry 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-H₂O
(4:1:5). The dried paper was sprayed with bro-
mothyl 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°, [α]D -36° (conc. 0.4 g in CHCl₃).
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-methylenecycloartenol, m.p.
120–121, [α]D⁰ -45° (conc. 0.3 g in CHCl₃), IR
(KBr) 3400, 1629, 1010, 890 cm⁻¹.

Fractions (10–12) eluted with benzene-chloro-
form (9:1) yielded nimbim which on crystallization
from methanol melted at 203–205°, [α]D⁰ +168
(conc. 1 g in CHCl₃) (lit.⁴ m.p. 201–204°,
[α]D⁰ +170°).

Fractions (13–15) eluted with benzene-chloro-
form (4:1) on repeated chromatography on silica
gel gave azadirone in gummy form, [α]D⁰ +23°
(conc. 0.55 g in CHCl₃) (lit.⁵ [α]D⁰ +26°),λ_max
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°, [α]D⁰ -40° (conc. 1 g
in CHCl₃), (lit.⁶ m.p. 218°, [α]D⁰ -44°),λ_max
225 nm (9200), IR (KBr) 1745, 1675,
885 cm⁻¹.

β-Sitosterol and 24-methylenecycloartenol were
the only two steroids obtained from the neem
exudate. Earlier, these steroids were isolated from
the leaves and heartwood of this plant⁶⁷. The
limonoids nimbim, 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 Aspergillus
flavus strains which elaborate aflatoxins (a well-
known carcinogen and mutagen) is a serious
hazard, especially in the case of species that are
indigenous to tropical regions. Various attempts to
eliminate aflatoxin production by using dimethyl-
 sulfoxide as a growth inhibitor have failed. In
order to prevent the spread of these fungi, it is
necessary to subculture the mold at regular intervals
of time to ensure that no aflatoxin is produced.

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; V-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 at
28 ± 2°C. After seven days the medium was filtered
and extracted with chloroform. Qualitative and quanti-
tative estimations 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