3.1 EXTRACTION AND FRACTIONATION OF THE EXTRACTS OF THE ROOT AND THE STEM BARKS OF AILANTHUS MALABARICA AND A. ALTISSIMA

To ensure exhaustive extraction of the plant material, repeated solvent extraction at room temperature was done, first, with petroleum ether followed by extraction of the defatted material with methanol. Solvents from all extracts and fractions were removed under reduced pressure using rotary vacuum evaporators to avoid any deterioration due to elevated temperature. Methanol extracts, after concentrating to a syrupy mass were partitioned between chloroform and water (containing 10% methanol).

Chloroform soluble portions of the methanol extracts were subjected to repeated chromatographic fractionation on silica gel columns which was monitored through TLC. Techniques of preparative TLC including centrifugally accelerated radial TLC were employed to achieve separation of components from the fractions where either the quantity was too small to put on column or separation through column chromatography was not successful. Only those fractions which showed clear separation on TLC were subjected to preparative TLC.

The petroleum ether extracts were also processed
for the isolation of the quassinoids and alkaloids but none of them yielded any quassinoid or alkaloid. Though all the fractions and subfractions were processed for the isolation of quassinoids and alkaloids but only successful attempts have been included in this manuscript.

3.2 ISOLATION OF QUASSINOIDs AND ALKALOIDS FROM THE ROOT AND THE STEM BARKS OF AILANTHUS MALABARICA AND A. ALTISSIMA

3.2.1 Ailanthus malabarica Root Bark

Six pure isolates, A.m./K₁ to A.m./K₆, were obtained from the chloroform soluble portion 'D₂' of the methanol extract of the root bark of A. malabarica. The fractionation of the chloroform soluble portion 'D₂' of the methanol extract through column chromatography yielded eight fractions (MR-1 to MR-8).

The fraction MR-2, eluted in chloroform containing 1% and 2% methanol, was again subjected to column chromatography using ethyl acetate as eluant. The preparative TLC of the subfraction MR-2.2 thus, obtained, resulted in the isolation of A.m./K₁ which was crystallized from a mixture of chloroform:methanol and recrystallized from acetone. Another subfraction, MR-2.4 from the same column on keeping in a mixture of chloroform:methanol yielded A.m./K₂ which was recrystallized from acetone.

The compound A.m./K₃, crystallized directly
from the fraction MR-3 eluted in chloroform containing 2% methanol, was purified by recrystallization from acetone.

The mother liquor left after separating A.m./K₃ from the fraction MR-3 was evaporated to dryness and subjected to repeated column chromatographic separations. The ether insoluble portion of the subfraction MR-3.1.2.2 from the second column was rechromatographed and the compound A.m./K₄ was isolated as precipitates from its subfraction MR-3.1.2.2.2, eluted in chloroform containing 2% methanol.

The compound A.m./K₅ was isolated from the subfraction MR-4.6 obtained by rechromatography of the fraction MR-4.

The crystalline compound A.m./K₆ was obtained directly from the fraction MR-5 eluted in chloroform containing 5% methanol.

3.2.2 *Ailanthus malabarica* Stem Bark

Three crystalline compounds, A.m./K₇ to A.m./K₉, were isolated from the chloroform soluble portion 'D₄' of the methanol extract. The initial column chromatographic fractionation of the chloroform soluble portion gave 10 fractions (MS-1 to MS-10).

The fraction MS-3, eluted in chloroform containing 1% methanol was rechromatographed over silica gel column and the subfraction MS-3.4 thus obtained
was again subjected to column chromatography. A residue settled in the subfraction MS-3.4.2 on keeping in a mixture of chloroform:methanol and the preparative TLC of this residue gave a crystalline compound A.m./K₇.

The subfraction MS-4.6, obtained by rechromatography of the fraction MS-4, on fractionation over chromatotron gave two crystalline compounds, A.m./K₈ and A.m./K₉.

3.2.3 *Ailanthus altissima* Root Bark

Four crystalline compounds, A.a./K₁ to A.a./K₄, were obtained from the chloroform soluble portion 'D₆' of the methanol extract. The chloroform soluble portion of the methanol extract 'D₆' was chromatographed over silica gel column which gave ten fractions (AR-1 to AR-10).

The fraction AR-6, eluted in chloroform containing 2% methanol, was rechromatographed over silica gel column using ethyl acetate as eluant. The subfraction AR-6.2 was once again subjected to column chromatographic separation and its subfraction AR-6.2.3, eluted in chloroform containing 1% and 2% methanol, yielded crystalline compound A.a./K₁ which was recrystallized from methanol. Another subfraction AR-6.2.5, eluted in chloroform containing 4% methanol, deposited yellowish brown precipitates which were crystallized from acetone to get A.a./K₂.
The fraction AR-7, eluted in chloroform containing 2% methanol was rechromatographed over silica gel column. The ether insoluble portion of the subfraction AR-7.3, eluted in chloroform containing 2% methanol yielded a crystalline compound A.a./K₃.

The crystalline compound A.a./K₄ was obtained directly from the fraction AR-9.

3.2.4 *Ailanthus altissima* Stem Bark

Three crystalline compounds, A.a./K₅ to A.a./K₇, were isolated from the chloroform soluble portion 'D₈' of the methanol extract. The chloroform soluble portion of the methanol extract was chromatographed over silica gel column which gave eight fractions (AS-1 to AS-8).

The compound A.a./K₅ was isolated from the subfraction AS-3.3 eluted in ethyl acetate. The subfraction AS-3.3 was obtained by rechromatography of the fraction AS-3, eluted in 1% methanol in chloroform.

Rechromatography of the fraction AS-5, eluted in chloroform containing 2% and 4% methanol resulted in the isolation of A.a./K₆ from one of its subfraction AS-5.3 eluted in chloroform containing 1% and 2% methanol.

The crystalline compound A.a./K₇ was isolated from the subfraction AS-6.4, eluted in chloroform containing 2% and 5% methanol obtained by rechromatography of the fraction AS-6.
3.3 CHARACTERIZATION AND IDENTIFICATION OF THE ISOLATED QUASSINOIDS AND ALKALOIDS FROM THE ROOT AND THE STEM BARKS OF AILANTHUS MALABARICA AND A. ALTISSIMA

3.3.1 Isolates of Ailanthus malabarica Root Bark

3.3.1.1 A.m./K₁

The crystalline compound A.m./K₁ having m.p. 158-159°C showed single spot on TLC (Rf 0.53 in chloroform:methanol 29:1 & Rf 0.54 in toluene:acetone:ethanol: ammonia 50:30:4:1.5). It gave strongly positive colour reaction with Dragendorff’s spray and fluoresced yellowish green under ultraviolet light. The UV spectrum in methanol exhibited absorption at 305, 302, 275, 258, 246 and 212 nm which was comparable to those reported for β-carbolines. In the mass spectrum the molecular ion peak was observed at m/z 226 (40%) and a base peak appeared at m/z 166 corresponding to the loss of a fragment of 60 mass units from the molecular ion peak. Other prominent peaks appeared at m/z 168 and m/z 140 indicative of β-carboline moiety which was also evident from its UV spectrum. The 360 MHz ¹H-NMR spectrum taken in CDCl₃ showed two multiplets, each for two aromatic protons at 8.15 and 7.60 attributed to protons at carbons C-5 to C-8 of β-carboline moiety indicating absence of any substitution on the aromatic ring A. The ortho coupled protons at C-3 and C-4 were seen as two doublets (J=5 Hz) at 8.58 and 7.34 respectively. The broad singlet at 9.92 was
assigned to proton at nitrogen. The observed absence of signal only for a proton at C-1 clearly indicated monosubstitution at C-1 on β-carboline moiety. The absorption corresponding to keto at 1750 cm\(^{-1}\) in the IR spectrum, singlet for three methoxyl protons at 64.13 in the \(^1\)H-NMR spectrum and indicated substitution of 59 mass units on β-carboline moiety in the mass spectrum confirmed the substituent as carbomethoxy (-COOCH\(_3\)). On the basis of the above observations, the isolate A.m./K\(_1\) was identified as 1-carbomethoxy-β-carboline (183). The comparison of our spectral data with that reported for this compound 197,278 confirmed our findings.

\[(183)\]

Its occurrence has already been reported from Ailanthus malabarica,\(^{197}\) A. altissima\(^{204}\) and Picrasma quassioides.\(^{278}\)

3.3.1.2 A.m./K\(_2\)

The isolate A.m./K\(_2\) was obtained as yellow needles with m.p. 153-154°C. The compound showed positive Dragendorff's test and single spot on TLC (Rf 0.49
in chloroform:methanol 29:1 & Rf 0.61 in toluene:acetone:ethanol:ammonia 50:30:4:1.5). On HPLC, it exhibited sharp peak with retention time 4.42 min on normal phase (Resolve 5 μ spherical silica) in chloroform (0.70 ml/min). The UV absorption at 368, 345, 298, 266, 258, 249 and 226 nm, the absorption at 1670 cm⁻¹ in the IR spectrum showing presence of α,β-unsaturated ketone and observed molecular ion peak at m/z 220 (100%) and consecutive loss of CO and HCN from the molecular ion in the mass spectrum were suggestive of canthin-6-one which was substantiated by identification of signals in its ¹H-NMR spectrum.

The 180 MHz spectrum taken in CDCl₃ showed pair of doublets (J=5 Hz) at δ 7.91 and δ 8.79 for H-1 and H-2 protons respectively. Another pair of doublets (J=10 Hz) at δ 7.99 and δ 6.95 represented protons at C-4 and C-5 respectively. The doublets at δ 8.62 and δ 8.05 (J=8 Hz) were observed for H-8 and H-11 respectively. The two protons at C-9 and C-10 appeared as triplets (J=8 Hz) at δ 7.67 and δ 7.49 respectively.

On the basis of above observations, A.m./K₂ was identified as canthin-6-one (180). This was further confirmed by getting superimposable IR spectrum and co-TLC with reference sample of canthin-6-one procured from the College of Pharmacy, University of Illinois at the Medical Centre, Chicago, U.S.A.
Canthin-6-one has not been isolated earlier from *Ailanthus malabarica*, however, it is a well known alkaloid from many other simaroubaceous plants viz. *A. altissima*, *A. excelsa*, *Hannoine klaineana*, *Brucea antidysenterica*, *Picrasma quassioides*.  

3.3.1.3 A.m./K$_3$

The crystalline compound A.m./K$_3$ having m.p. 277-278°C showed single spot on TLC (Rf 0.59 in chloroform:methanol 9:1 & Rf 0.67 in ethyl acetate:isopropanol:ammonia 45:35:15) and sharp peak in HPLC with retention time 2.71 min on normal phase (Resolve 5µ spherical silica) using chloroform (0.50 ml/min) as eluant. On TLC, the compound developed faint orange colour with Dragendorff's spray and produced brick red colour on heating after spraying with 60% sulphuric acid. The compound failed to develop precipitates when tested with Mayer's reagent and showed absence of nitrogen in its molecule in the Lassaigne sodium test for nitrogen. These observations indicated that the compound gives false positive test with Dragendorff's reagent as reported with a number of non-alkaloidal constituents.
The mass spectrum showed molecular ion peak at m/z 478 and other prominent fragments appeared at m/z 394, 377, 319, 247, 151, 85 and 57. The fragments at m/z 247 (XXIII) and m/z 151 (XXIV) are characteristic of quassinoids, the former results from the cleavage of C8-C14, C11-C12 and C7-0 bonds and the latter from C10-C9 and C6-C7 bond cleavage. The quassinoid nature of A.m./K₃ was also indicated by the IR spectrum which showed intense absorption in the region of hydroxyls (3400-3200 cm⁻¹), presence of δ-lactone (1750 cm⁻¹) and α, β-unsaturated carbonyl (1675 cm⁻¹). Absorption at 239.5 nm in the UV spectrum indicated α,β-unsaturated ketone in ring A of the quassinoid. The 180 MHz ¹H-NMR spectrum taken in pyridine-d₅ showed two non-equivalent protons of epoxymethano bridge as two doublets (J=8.6 Hz) at δ4.06 and δ3.76, typical of the quassinoids with bridge in ring C. A singlet for vinylic H-3 proton was observed at δ6.02. A sharp singlet at δ3.18 was easily recognized for H-9 proton. Other two singlets at δ4.08 and δ4.69 were identified for H-7 and H-1.
respectively. A 3H doublet at $\delta 1.19$ ($J=7.2$ Hz) and 3H singlets at $\delta 1.75$ and $\delta 1.44$ were assigned to protons of methyls at C-13, C-4 and C-10 respectively. A doublet for $\alpha$-axial proton at C-15 with coupling constant of 10.8 Hz in the downfield region at $\delta 6.21$ was indicative of ester moiety at C-15 which was also suggested by fragment at m/z 394 in the mass spectrum resulting from shift of $\alpha$-hydrogen of ester chain to oxygen at C-16 and simultaneous cleavage of O-C bond (Scheme 2).

![Scheme 2](image)

The fragments at m/z 85 and m/z 57 in the mass spectrum suggested a five carbon ester moiety
on quassinoid skeleton which was also supported by the loss of a fragment of 101 mass units from the molecular ion. The structure, α-methylbutyrate, for the ester moiety was deduced from the $^1$H-NMR spectrum which showed signals for protons of primary methyl (δ 0.98, t; J=7.7 Hz) and secondary methyl (δ 1.24, d; J=7.7 Hz) in addition to the already assigned methyl protons signals of the basic quassinoid nucleus and mass spectrum which showed peak at m/z 377 corresponding to the loss of the ester moiety (Scheme 2).

On the basis of the above observations and their comparison with the spectral data from the literature the isolate A.m./K^3 was identified as ailanthinone (95). Ailanthinone is being reported here for the first time from Ailanthus malabarica, however, it has earlier been reported from Pierreodendron kerstingii, Simarouba versicolor, Ailanthus excelsa, Hannoa undulata and Odyendyea gabonensis.
3.3.1.4 A.m./K₄

The isolate A.m./K₄ obtained as white precipitates showed a single spot on TLC (Rf 0.61 in chloroform: methanol 25:5). The mass spectrum of the compound showed molecular ion peak at m/z 476 and prominent fragments at m/z 458, 432, 392, 330, 255, 247, 165, 151, 85 and 57. Some of these fragments like m/z 247 and m/z 151 are common in quassinoids which have α,β-unsaturated ketone in ring A and hemiketal bridge in ring C.¹⁵²

The ¹H-NMR spectrum taken in a mixture of CDCl₃ and CD₃OD showed the presence of H-3 vinylic proton as a broad singlet at δ6.19. The magnetically non-equivalent, mutually coupled protons at the hemiketal bridge carbon were seen as a pair of doublets (J=9.7 Hz) at δ3.93 and δ3.56. A sharp singlet, characteristic of quassinoids with uncoupled proton at C-9 appeared in its normal range at δ3.05. The signals for methyl protons at C-4 and C-10 were observed as singlets at δ2.02 and δ1.20 respectively. The absence of signal for C-13 methyl protons and instead appearance of a pair of doublets (J=2.1 Hz) at δ5.40 and δ5.19 confirmed the exo-methylene group at C-13. A doublet for α-axial proton at C-15 in the downfield region at δ5.60 (J=14.9 Hz) was suggestive of ester moiety at C-15 which was also supported by the observed fragments at m/z 85 (C₄H₉-C≡O⁺) and m/z 57 (C₄H₉⁺) in its mass spectrum.
A 3H triplet for primary methyl at δ 0.96 (J = 8.6 Hz) and 3H doublet at δ 1.19 (J = 7.2 Hz) for secondary methyl and loss of 84 mass units (C₄H₈CO) from the molecular ion peak to produce peak at m/z 392 established the α-methylbutyrate ester moiety at C-15. The triplet at δ 4.61 (J = 3.6 Hz) and two singlets at δ 4.16 and δ 4.06 were the other signals assigned for H-7, H-12 and H-1 protons respectively.

The above spectral observations and their comparison with the known quassinoid isolated earlier from *Pierreodendron kerstingii*¹⁰⁵ confirmed it to be 13,18-dehydroailanthinone (110). It is being reported from the *Ailanthus malabarica* for the first time.

### 3.3.1.5 A.m./K₅

The crystalline compound A.m./K₅ having m.p. 280-282°C showed single spot on TLC (Rf 0.45 in chloroform:methanol 25:5). Its IR spectrum suggested presence of hydroxyl (3400 cm⁻¹) and δ-lactone (1740 cm⁻¹). The mass spectrum exhibited highest mass peak at m/z 452 and other fragments were observed at m/z 434, 381,
364, 265, 247, 219, 151, 135, 83, 55 and 43. The fragments at m/z 247, 151 and 135 are characteristic of quassinoids and the ions at m/z 151 and m/z 135 are observed in quassinoids possessing α, β-unsaturated ketone in ring A. Its 360 MHz $^1$H-NMR spectrum taken in pyridine-$d_5$ also confirmed the quassinoid nature of the compound with the identification of characteristic signals of methylene protons of hemiketal bridge, vinylic proton at C-3, proton at terminal carbon of δ-lactone and uncoupled proton at C-9. Taken together, these observations indicated the quassinoid nature of the compound, presence of α,β-unsaturated ketone in ring A and hemiketal bridge between C-8 and C-11 in ring C. The presence of α,β-unsaturated ketone in ring A was also supported by absorption in the UV spectrum at 215 nm. Its $^1$H-NMR spectrum showed a singlet at δ 5.96 for vinylic proton at C-3. The proton at C-1 was observed as a broad singlet at δ 4.90 and 3H singlet for C-4 methyl protons observed at δ 2.54. These observations confirmed the structure (IX) for the ring A.

(IX)

The singlet at δ 4.98 was attributed to proton at C-7 and a sharp singlet at δ 3.40 was assigned to
H-9 proton. The 3H singlet at δ1.52 was assigned to methyl protons at C-10. The pair of doublets (J=10.4 Hz) at δ3.98 and δ3.90 was assigned to non-equivalent methylene protons of hemiketal bridge in ring C. The triplet at δ4.03 (J=5.7 Hz) was assigned to proton at C-12 and the methyl protons at C-13 were responsible for a 3H doublet at δ1.39 (J=8.6 Hz).

In ring D, the C-15 proton was observed as a doublet at δ6.31 (J=14 Hz) indicating an ester moiety at C-15. The fragments at m/z 83 (C₄H₇-C=O⁺) and m/z 55 (C₄H⁺) observed in its mass spectrum and signals for primary methyl protons (δ1.26, t; J=7 Hz) and another singlet at δ1.74 for methyl protons also indicated the presence of an ester moiety in the quassinoid. It was not possible to pick up the correct molecular ion peak in the mass spectrum though it was taken twice and for this reason the structure of A.m./K₅ could not be confirmed. However, these observations enable us to propose the following partial structure (XXV)
and we speculate the compound to be in glaucarubinone series.

3.3.1.6 A.m./K₆

The crystalline compound A.m./K₆ having m.p. 285-287°C showed single spot on TLC (Rf 0.40 in chloroform:methanol 9:1). On HPLC, it exhibited sharp peak with retention time 2.65 min on reverse phase (Resolve 5µ spherical C18) using methanol (0.50 ml/min) as a mobile phase. The IR spectrum showed presence of hydroxyl (3425 cm⁻¹) and keto (1750 cm⁻¹). Examination of its 360 MHz ¹H-NMR spectrum taken in pyridine-d₅ indicated its quassinoid nature with the identification of signals for vinylic proton at C-3 (δ5.72, d; J=1.1 Hz), non-equivalent, mutually coupled methylene protons of hemiketal bridge (δ4.17 & δ3.84, d; J=8.6 Hz), proton at C-9 (δ3.12, s) and protons of methyls at C-4 (δ1.66, s), C-10 (δ1.54, s) and C-13 (δ1.31, d; J=7.2 Hz). The singlet at δ4.66 was attributed to H-7 and the singlet at δ4.55 represented H-2. The triplet at δ4.01 which changes to singlet in D₂O exchange was assigned to H-12. The proton at C-1 appeared as doublet at δ3.82 (J=8.6 Hz). The 6α and 6β protons appeared as undistinguishable multiplets in the region of δ1.81 to δ2.05. The signals at δ10.01 (s), δ7.28 (d, J=5 Hz), δ6.32 (d, J=9.7 Hz) and δ4.99 (s) which disappeared in D₂O exchange represented the hydroxyls in the molecule.
The doublet for α-axial H-15 proton in the downfield region at δ 6.40 (J=12.2 Hz) and signals for primary methyl protons (δ1.00, t; J=7.5 Hz) and secondary methyl protons (δ1.22, d; J=7.2 Hz) suggested an ester chain at C-15. The mass spectrum showed prominent fragments at m/z 478, 462, 361, 231, 135, 85 and 57. The peak at m/z 231 (8%) is generated with the loss of water molecule from the fragment at m/z 249 (15) which itself is characteristic of quassinoids resulting from cleavage of C8-C14, C11-C12 and C7-O bonds (Scheme 3). The ion at m/z 135 formed with the cleavage of C9-C10 and C6-C7 bonds is also characteristic of quassinoids.

The intense fragments at m/z 85 (C₄H₉-C=O⁺) and m/z 57 (C₄H₇⁺) taken together with signals for primary and secondary methyl protons at δ1.00 (t, J=7.5 Hz) and δ1.22 (d, J=7.2 Hz) respectively in its ¹H-NMR spectrum confirm the α-methyl butyrate ester moiety at C-15. The identification of H-2 proton signal (δ4.55) and lack of absorption in the region of 220-240 nm in the UV spectrum showed the absence of keto at C-2 in ring A.
These spectral observations led to the identification of isolate A.m./K\textsubscript{6} as excelsin (77) which was confirmed by comparing our findings with those reported in literature.\textsuperscript{78,79,280}

Though the mass spectrum of A.m./K\textsubscript{6} was taken twice but we failed to pick up the molecular ion peak at m/z 480 which has earlier been reported for excelsin.\textsuperscript{78,79} A similar report on the mass spectrum indicating absence of molecular ion peak, as observed by us, has also been published.\textsuperscript{280}

The occurrence of excelsin is being reported from the Ailanthus malabarica for the first time though this quassinoid has earlier been reported from A. excelsa,\textsuperscript{79} Odyendyea gabonensis\textsuperscript{78} and Pierreodendron kerstingii.\textsuperscript{74}

3.3.2 Isolates of Ailanthus malabarica Stem Bark

3.3.2.1 A.m./K\textsubscript{7}

The yellow crystalline compound A.m./K\textsubscript{7} having m.p. 153-154°C showed single spot on TLC (Rf 0.49 in chloroform:methanol 29:1 & Rf 0.61 in toluene:acetone:ethanol:ammonia 50:30:4:1.5). It fluoresced blue under
ultraviolet light and gave positive colour reaction with Dragendorff's spray. The UV spectrum exhibited absorption at 368, 345, 298, 266, 258, 249 and 226 nm. Its UV spectrum and TLC showed it to be identical with A.m./K₂ isolated from the root bark of Ailanthus malabarica and characterized as canthin-6-one. This was further confirmed by having superimposable IR spectrum and co-TLC with canthin-6-one which led to the identification of A.m./K₇ as canthin-6-one.

3.3.2.2 A.m./K₈

The colourless crystalline compound A.m./K₈ having m.p. 156°C showed single spot on TLC (Rf 0.40 in chloroform:methanol 19:1 & Rf 0.55 in toluene:acetone: ethanol:ammonia 50:30:4:1.5), produced sky blue fluorescence under ultraviolet light and gave positive colour reaction with Dragendorff's spray. In the mass spectrum it showed molecular ion peak at m/z 256 (100%). In the UV spectrum it showed absorption at 349, 332, 283, 272 and 242 nm which suggested a β-carboline nucleus.¹⁹⁷,²₀₀

The 360 MHz ¹H-NMR spectrum taken in CDCl₃ showed 3H sharp singlets at 64.11 and 64.03 which suggested two methoxyl substituents on β-carboline nucleus. The triplet for primary methyl protons at 61.44 (J=8.2 Hz) and quintet for methylene protons at 63.09 (J=8.2 Hz) confirmed the third substituent as ethyl and these three substituents were in accordance with the observed mole-
cular ion peak in the mass spectrum. This is also supported by the repeated loss of methyls and loss of ethyl from the molecular ion.

The appearance of H-3 proton in the upfield region at $\delta 7.99$ from the normal value of $\delta 8.45$ indicated that one of the methoxyls is substituted at C-4. The ethyl group as second substituent on ring C at C-1 is supported by the comparison of chemical shift values of methylene and methyl protons with those given for 1-ethyl-4-methoxy-β-carboline. The second methoxyl must be substituted at C-8 was shown by identifying the signals for H-5, H-6 and H-7 protons at $\delta 7.90$ (d, $J=9.3$ Hz), $\delta 7.21$ (t, $J=9.3$ Hz) and $\delta 6.97$ (d, $J=9.3$ Hz) respectively. The NH proton was observed as a broad singlet at $\delta 8.38$.

On the basis of the above spectral observations and their comparison with the spectral data from literature, the isolate A.m./Kg was identified as 4,8-dimethoxy-1-ethyl-β-carboline (184). This alkaloid has previously been reported from Picrasma quassioides, Aeschirion crenata and Ailanthus malabarica.

\[
\begin{align*}
\text{OCH}_3 & \quad \text{CH}_2\text{CH}_3 \\
\text{OCH}_3 & \quad \text{CH}_2\text{CH}_3
\end{align*}
\]

(184)
3.3.2.3 A.m./K9

The yellow crystalline compound A.m./K9 having m.p. 221°C showed single spot on TLC (Rf 0.55 in chloroform:methanol 25:5 & Rf 0.46 in toluene:acetone:ethanol: ammonia 50:30:4:1.5). On HPLC, it produced sharp peak with retention time 2.75 min on reverse phase (Resolve 5 μ spherical C18) using methanol (0.40 ml/min) as an eluant. It fluoresced blue under ultraviolet light and gave positive test for alkaloids. The IR spectrum showed presence of hydroxyl (3350 cm⁻¹), conjugated carbonyl (1670 cm⁻¹) and suggested its aromatic nature (740 cm⁻¹). Its UV spectrum showed absorption at 376, 359 and 283 nm.

The above characteristics indicated its canthinone nature and this was supported by its mass spectrum which showed peaks corresponding to the successive loss of CO and HCN from the molecular ion peak. The IR spectrum which showed absorption corresponding to hydroxyl and molecular ion peak at m/z 236 in the mass spectrum suggested the substitution of hydroxyl on the canthinone nucleus.

The examination of its 360 MHz 1H-NMR spectrum taken in pyridine-d₅ showed presence of two doublets (J=9 Hz) at δ8.97 and δ8.53 corresponding to H-8 and H-11 proton respectively. The two triplets (J=9 Hz) at δ7.67 and δ7.57 were assigned to H-9 and H-10 protons.
respectively, which indicated unsubstituted aromatic ring A. The pair of doublets (J=11.5 Hz) at δ 8.11 and δ 6.90 represented protons at C-4 and C-5 respectively. The appearance of H-2 as a singlet at δ 8.81 in the absence of proton at C-1 fixed the position of hydroxyl at C-1.

From the above observations the isolate A.m./K9 was identified as 1-hydroxycanthin-6-one (185) which was confirmed by comparison of the spectral data with its earlier report from Ailanthus giraldii.202

3.3.3 Isolates of Ailanthus altissima Root Bark

3.3.3.1 A.a./K1

The yellow crystalline needles of A.a./K1 having m.p. 152-153°C showed single spot on TLC (Rf 0.49 in chloroform:methanol 29:1 & Rf 0.61 in toluene:acetone:ethanol:ammonia 50:30:4:1.5). It fluoresced blue under ultraviolet light and gave positive test for alkaloid. HPLC of the isolate gave a sharp peak with retention time 4.42 min on normal phase (Resolve 5μ spherical
silica) using chloroform (0.70 ml/min) as eluting solvent. Its UV spectrum exhibited absorption at 368, 345, 298, 266, 258, 249 and 226 nm. These observations indicated its identical nature with A.m./K₂ isolated from the root bark and A.m./K₇ isolated from the stem bark of Ailanthus malabarica. The isolate A.a./K₁ was thus identified as canthin-6-one (180) and was confirmed by getting superimposable IR spectrum and co-TLC with reference compound.

3.3.3.2 A.a./K₂

The yellow crystalline compound A.a./K₂ showed m.p. 251°C and single spot on TLC (Rf 0.64 in chloroform: methanol 9:1 and Rf 0.43 in toluene:acetone:ethanol: ammonia 50:30:4:1.5), fluoresced parrot green under ultraviolet light and developed orange colour with Dragendorff's spray indicating its alkaloidal nature. In the UV spectrum it exhibited absorption at 365, 278 and 245 nm and the IR spectrum showed absorption at 1670 cm⁻¹ for conjugated carbonyl, prominent peak at 1235 cm⁻¹ suggesting presence of N-O bond in the compound and absorption at 780 and 750 cm⁻¹ indicating its aromatic nature. Its MS exhibited molecular ion peak at m/z 236 (100%) and a peak at m/z 208 corresponding to loss of CO from the molecular ion. Taken together, these characteristics indicated canthinone moiety.

Its 360 MHz ¹H-NMR spectrum taken in CDCl₃
showed pair of doublets ($J = 6.5 \text{ Hz}$) at $\delta 7.91$ and $\delta 8.36$ for protons at C-1 and C-2 respectively. The two olefinic protons at $\alpha$- and $\beta$-carbon to carbonyl appeared as pair of doublets ($J = 10 \text{ Hz}$) at $\delta 6.93$ and $\delta 8.37$ respectively. The two doublets ($J = 7.5 \text{ Hz}$) at $\delta 8.58$ and $\delta 8.02$ and two triplets ($J = 7.5 \text{ Hz}$) at $\delta 7.64$ and $\delta 7.53$ were attributed to four aromatic protons on ring A at C-8, C-11, C-9 and C-10 respectively.

The observed signals in the $^1\text{H-NMR}$ spectrum for all the protons of canthin-6-one nucleus ruled out any substitution on the basic skeleton and addition of 16 mass units as indicated by the molecular ion peak was then possible by linkage of either nitrogen to oxygen through coordinate bond which was confirmed by observed absorption at $1235 \text{ cm}^{-1}$ in the IR spectrum for N-O stretching absorption. To confirm the position of N-oxide, its $^1\text{H-NMR}$ spectral data was compared with that of canthin-6-one. Both the spectras were run in CDCl$_3$. The signal for the proton at C-2 in A.a./K$_2$ showed a shift of $\delta 0.43$ indicating the N-oxide to be located at position 3.

Based on the above discussion, structure (186), canthin-6-one-3N-oxide was proposed for the crystalline compound A.a./K$_2$. Canthin-6-one-3N-oxide was also obtained by oxidation of canthin-6-one with m-chloroperbenzoic acid which proved to be identical with A.a./K$_2$.
through co-TLC and IR spectrum thus confirming the structure (186) for A.a./K₂.

![Structure](186)

3.3.3 A.a./K₃

The crystalline compound A.a./K₃ having m.p. 278-280°C showed single spot on TLC (Rf 0.47 in chloroform:methanol 9:1) which developed yellow colour on heating after spraying with 60% sulphuric acid. It showed lack of notable UV absorption.

The mass spectrum showed molecular ion peak at m/z 364 and other prominent fragments were observed at m/z 346, 318, 274, 187, 135, 55 and 41. Due to the poor solubility in CDCl₃, its ¹H-NMR spectrum was taken in pyridine-d₅. The poor quality spectrum made it difficult the assignment of signals for different protons, nevertheless, it indicated the presence of quassinoid skeleton and the main signals which could be interpreted were doublets at δ0.69 (J=5.5 Hz) for 4-CH₃ protons, δ0.99 (J=7.2 Hz) for 13-CH₃ protons and two singlets at δ1.46 and δ1.49 attributed to C-8 and C-10 methyl
protons respectively. The H-7 proton was observed as a broad singlet at δ4.30. The doublet at δ4.65 (J=12 Hz) which sharpened in D2O exchange was attributed to proton at C-11 that couples with proton at C-9 which itself showed doublet at δ2.63. The multiplet at δ5.07 was assigned to proton at C-2.

Though all these spectral observations were not enough to propose any final structure but these indicated the possibility of being this compound as amarolide (56). The sample of A.a./K3 tentatively identified as amarolide was sent to Department of Chemistry, University of Tokyo, Tokyo, Japan for their opinion on the structure which they confirmed as amarolide by comparison with the authentic sample. Amarolide has earlier been reported from Castela nicholsoni 56 and C. texana57 in addition to its first report from Ailanthus glandulosa.55

3.3.3.4 A.a./K4

The isolate A.a./K4 was obtained as crystalline
cubes having m.p. 234-235°C. It showed single spot on TLC (Rf 0.62 in chloroform:methanol 25:5) which developed yellow colour changing to brown on heating after spraying with 60% sulphuric acid. On HPLC, it exhibited peak with retention time 2.66 min on normal phase (Resolve 5μ spherical silica) using mixture of chloroform:methanol 9:1 (0.70 ml/min) as eluting solvent. The UV spectrum exhibited absorption at 240 nm indicating conjugated carbonyl which was also supported by observed absorption at 1690 cm⁻¹ in its IR spectrum. Other features indicated in the IR spectrum were the presence of hydroxyl (3300 cm⁻¹) and another carbonyl (1720 cm⁻¹). The mass spectrum showed intense molecular ion peak at m/z 376 (54%) and other major fragments were observed at m/z 264, 248, 247, 151 and 135 which suggested its quassinoid nature. The 250 MHz ¹H-NMR spectrum exhibited a quintet at δ6.18 (J=1.4 Hz) attributed to olefinic proton at C-3 showing that it coupled with vinylic methyl protons at C-4. The H-1 proton was observed as a singlet at δ4.04. The two magnetically non-equivalent protons at hemiacetal bridge carbon in ring C were observed as two doublets (J=8.5 Hz) at δ3.92 and δ3.55. The appearance of two singlets at δ5.38 and δ5.28 for one proton each and the simultaneous absence of usual doublet for methyl at C-13 of quassinoid skeleton indicated the presence of exo-methylene moiety at C-13 in ring C. The triplet
at δ 4.53 (J=2.8 Hz) was assigned to proton at C-7. The 6α-proton coupled to 6β, 5α and 7β protons with coupling constants of 14.7 Hz, 2.7 Hz and 2.7 Hz respectively produced signal centered at δ 2.33. The signal for 6β-proton overlapped under signal for 4-methyl protons at δ 2.04. The singlet for H-9 was observed at δ 2.73. The α proton at C-15 appeared as double doublet (J=20 Hz & 15 Hz) at δ 3.09. The signals for protons at C-5, C-14 and β-proton at C-15 could not be ascertained owing to the overlapping of various signals for these protons in the region of δ 2.65 to δ 2.90.

On the basis of the above spectral details, A.a./K₁ was identified as ailanthone (109) which was then confirmed by getting superimposable IR spectrum and through co-TLC with the authentic sample of ailanthone.

3.3.4 Isolates of Ailanthus altissima Stem Bark

3.3.4.1 A.a./K₅

The yellow crystalline compound A.a./K₅ having m.p. 152-153°C showed single spot on TLC (Rf 0.49 in chloroform:methanol 29:1 and Rf 0.61 in toluene:acetone:...
ethanol:ammonia 50:30:4:1.5). It gave positive test for alkaloids and exhibited UV absorption at 368, 345, 298, 266, 258, 249 and 226 nm. These preliminary observations showed its similarity with canthin-6-one (180) which was confirmed by its IR spectrum and co-TLC with the authentic sample of canthin-6-one.

3.3.4.2 A.a./K_6

The crystalline compound A.a./K_6 having m.p. 235-236°C showed single spot on TLC (Rf 0.62 in chloroform:methanol 25:5) which developed yellow colour changing to brown on heating after spraying with 60% sulphuric acid. In the UV spectrum it exhibited absorption at 240 nm. These observations resembled those of A.a./K_4 identified as ailanthone (109). The final confirmation of A.a./K_6 as ailanthone was made by comparing its IR spectrum and co-TLC with reference sample of ailanthone.

3.3.4.3 A.a./K_7

The crystalline compound A.a./K_7 showed m.p. 260-263°C. It gave single spot on TLC (Rf 0.60 in chloroform:methanol 25:5) which developed yellow colour changing to dark brown on heating after spraying with 60% sulphuric acid. On HPLC, it produced peak with retention time 4.10 min on normal phase (Resolve 5μ spherical silica) in chloroform:methanol 9:1 (0.70 ml/min). In the UV spectrum maximum absorption was observed at 279 nm. The IR spectrum showed the presence of hydroxyl (3500-3200 cm^{-1})
and carbonyls (1770 cm$^{-1}$ & 1690 cm$^{-1}$). The mass spectrum exhibited molecular ion peak at m/z 362 and other prominent fragments at m/z 331, 265, 247, 235, 219, 201 and 189. These characteristics of A.a./K$_7$ indicated its similarity with shinjulactone B which was confirmed by the identification of the signals in the $^1$H-NMR spectrum.

The 270 MHz spectrum taken in pyridine-d$_5$ showed singlet at $\delta$ 6.08 for proton at C-5 and triplet (J=1.5 Hz) at $\delta$ 5.89 was assigned to proton at C-3. The H-7 proton appeared as doublet at $\delta$ 5.19 (J=6 Hz). The two hydroxymethyl protons (CH$_2$OH) appeared as two doublets (J=11.5 Hz) at $\delta$ 4.04 and $\delta$ 3.87. The two multiplets at $\delta$ 3.27 and $\delta$ 3.19 were assigned to H-13 and H-14 respectively. The two protons at C-15 appeared as double doublets at $\delta$ 2.89 and $\delta$ 2.65. The $\delta$ $\alpha$ and $\delta$ $\beta$ protons appeared as two multiplets in the region of $\delta$ 2.23 to $\delta$ 2.08. The 4-CH$_3$ and 10-CH$_3$ protons appeared as singlets at $\delta$ 1.93 and $\delta$ 1.61 respectively. The doublet at $\delta$ 1.07 (J=7 Hz) was attributed to C-13 methyl protons.
The isolate A.a./K7 was tentatively identified as shinjulactone B (69), which was confirmed through correlated spectroscopy (COSY) and comparison with authentic sample from Department of Chemistry, University of Tokyo, Tokyo, Japan.