Analysis of Insecticidal *Azadirachta indica* A. Juss. Fractions

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As a result of chemical investigation on the ethanolic extract of fresh fruit coatings of *Azadirachta indica* A. Juss. (neem), twenty-seven compounds were identified in non-polar to less polar fractions which showed pesticidal activity determined by WHO method against *Anopheles stephensi* Liston. These identifications were basically made through GC-EIMS and were further supported by other spectroscopic techniques, including ^{13}C NMR, UV and FTIR as well as retention indices. Thus sixteen *n*-alkanes, **1–16**; three aromatics 2,6-*bis*-(1,1-dimethylethyl)-4-methyl phenol (**17**), 2-(phenylmethylene)-octanal (**20**), 1,2,4-trimethoxy-5-(1*Z*-propenyl)-benzene (**27**); three benzopyranoids 3,4-dihydro-4,4,5,8-tetramethylcoumarin (**18**), 3,4-dihydro-4,4,7,8-tetramethylcoumarin-6-ol (**19**), 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[*g*]-2-benzopyran (**22**); one sesquiterpene methyl-3,7,11-trimethyl-2*E*,-6*E*,10-dodecatrienoate (**21**); three esters of fatty acids methyl 14-methyl-pentadecanoate (**23**), ethyl hexadecanoate (**24**), ethyl 9*Z*-octadecenoate (**25**) and one monoterpene 3,7-dimethyl-1-octen-7-ol (**26**) were identified. Except **6**, **8**, **24** and **25** all these compounds were identified for the first time from the pericarp and fifteen of these, **1–3**, **7**, **9**, **10**, **17–23**, **26**, **27**, are hitherto unreported previously from any part of the tree. Although this tree is a rich source of various natural products, it is the first report of identification of mono- and sesquiterpenes **26** and **21** and a potent antioxidant, **17**.

Key words: Azadirachta indica, Fruit Coats, *Anopheles stephensi*

Introduction

Azadirachta indica A. Juss. (syn. *Melia indica* Brandis; *Melia azadirachta* Linn.) known in common as neem (Urdu) and Indian lilac (English), belongs to the family Meliaceae (order Rutales). Neem is native to all of South, particularly Southeast Asia including Pakistan. It grows in tropical to subtropical regions, semiarid to wet tropical regions, and from sea level to about 700 m elevation. The ripe, ellipsoidal drupe (fruit) measures up to almost 2 cm in length and comprises sweet pulp and a seed covered in a smooth, yellow or yellowish green coating (Vietmeyer, 1992).

The people of South Asia know neem very well since very old times. Medicinal uses of various parts of neem tree are manifold. Its different parts are highly reputed in folklore and traditional system of medicine for the treatment of a variety of human aliments, particularly against the diseases of bacterial and fungal origin. It has been credited with insecticidal and repellant properties in this region (Vietmeyer, 1992; Schmutterer, 1995).

A variety of triterpenoids have been reported from neem (Akhila and Rani, 1999; Siddiqui *et al.*, 1999, 2000a,b, 2002; Siddiqui and Rasheed, 2001; Govindachari *et al.*, 1999; Jarvis *et al.*, 1999; Luo *et al.*, 2000). Various non-terpenoidal constituents have also been identified by different groups. These included hydrocarbons, aromatics, phenolics, coumarins, isocoumarins, flavones, fatty acids and their esters, sulfides, *etc.* (Akhila and Rani, 1999; Ali *et al.*, 1996; Kaushik and Vir, 2000; Sharma *et al.*, 1998; Siddiqui *et al.*, 1988, 1992).

The pesticidal activity of neem botanicals (chemical preparations of plant origin), including that of triterpenoids, neem oil and fractions containing volatiles against a variety of house and crop insects has remained the subject of interest since decades. Mosquitoes, carriers for certain diseases are among these (Ascher, 1997; Khan *et al.*, 1999; Tariq *et al.*, 2001, 2002; Naqvi *et al.*, 1994; Dhar *et al.*, 1996; Siddiqui *et al.*, 1999, 2000a,b, 2002; Mulla and Su, 1999; Vietmeyer, 1992).

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Experimental

Plant material

Fresh ripe neem (*Azadirachta indica* A. Juss.) fruits (50 kg) were collected from the Karachi region in the month of July and identified by Prof. Dr. S. I. Ali, Department of Botany, University of Karachi. A voucher specimen (NM-1) has been deposited in the herbarium, Department of Botany, University of Karachi.

Extraction

The fruits were separated manually into fruit coats and seeds and freed of the pulp. The uncrushed fresh seeds (27 kg) and coatings (23 kg) were extracted with EtOH (5 ×) at room temperature, and concentrated under reduced pressure. The syrupy extract of the fruit coats (RB-b) following the pesticidal activity-guided isolation against *Anopheles stephensi*, ultimately furnished thirteen fractions marked as 'A' through 'M' (Siddiqui *et al.*, 2000a; Siddiqui and Rasheed, 2001).

The portion eluted with 100% petroleum ether by vacuum liquid chromatography, designated as fraction 'A', was further purified on dry silica column to remove any oxygenated compound. The adsorbed material was recovered with EtOAc and combined with the next fraction 'B'. Fraction 'A' was concentrated at room temperature by bubbling nitrogen, to provide 300 mg of a light yellow volatile oil. The quantitative and qualitative analysis of fraction, resulted in the identification of sixteen hydrocarbons, **1–16** (Table I).

Fraction 'B' (2.5 g), the next eluate of VLC [petroleum ether/EtOAc 99:1 to 92:8 eluted with 1% gradient (8 fractions of 200 ml each)], resulted in the identification and quantitation of nine compounds. These included two aromatics, **19**, **22**, three benzopyranoids, **20**, **21**, **24**, and one sesquiterpene ester, **23**. Three esters of fatty acids, **25–27**, were also identified (Table II, Fig. 1).

Fraction 'C' (6.5 g) was a reddish brown thick gummy residue obtained on combining the petroleum ether/EtOAc 92:8 to 86:14 [1% gradient (7 fractions of 500 ml each)] and 85:15 to 70:30 [5% gradient (4 fractions of 2 l each)] eluates. Its analysis resulted in the identification and quantitation of five compounds. Three of these were the esters of fatty acids **25–27** identified in fraction 'B' whereas the two others were identified as a monoterpene alcohol **28** and an aromatic constituent **29**.

Instrumentation and identification

Gas chromatography using FID, was carried out on a Shimadzu gas chromatograph GC-17A hooked with Shimadzu Class GC-10 software and equipped with a less polar capillary column SPB-5® (30 m × 0.53 mm ID × 0.50 μm film thickness of 5% phenyl/95% methyl silicone). The analyses were performed with an initial temperature of 35 °C for 2 min, then ramped with 3 °C/min to a final temperature of 220 °C with final time 50 min (program A). Injector with splitting ratio of 1:60 was set at 250 °C and FID at 270 °C. Carrier and make up gas was nitrogen with a flow of 1.4 and 40 ml/min at a pressure of 0.3 and 1.6 kg/cm^2, respectively. Kovats retention indices were also calculated (Kovats, 1958).

For GC-EIMS experiments a Hewlett-Packard 5890 gas chromatograph was combined with a Jeol, JMS-HX 110 mass spectrometer operating in EI mode with ion source at 270 °C and electron energy at 70 eV. Injector was set at 270 °C with splitting ratio 1:30. Analyses were performed on the aforementioned program A as well as on another GC cycle (program B) on an equivalent column HP-5® (25 m × 0.22 mm and 0.25 μm film thickness), in order to obtain a better chromatogram and mass spectrum of poor broad late eluting peaks in program A as follows; carrier gas was helium at a pressure of 1.4 kg/cm^2. The column was kept initially at a temperature of 60 °C for one min, raised to a final temperature of 240 °C at a rate of 8 °C/min with final holding time 30 min. Mass spectral survey was performed using MS-libraries (NIST Mass Spectral Search Progam, 1998; GC-MS Library of Shimadzu, 1996).

^{13}C NMR spectra of fractions were recorded in $CDCl_3$ on a Bruker Aspect 3000 AM-300 spectrometer operating at 75 MHz. The chemical shifts are recorded in ppm (δ). Ultraviolet absorbance was measured in CH_3OH, on Hitachi U-3200 UV-visible spectrophotometers. Infrared transmissions were recorded on ATR using Bruker Vector 2000 FTIR spectrophotometer hooked with Opus software, version 3.0.

Physical and spectral data of fraction 'A'

Light yellow volatile oil (300 mg). UV (CH_3OH) λ_{max}: Transparent until solvent cut-off. – FTIR ($CHCl_3$): ν_{max} = 2953, 2923, 2853 (CH str.), 1461, 1377 (CH_3) cm^{-1}.

Characterization of constituents

n-Pentadecane (**1**): GC-EIMS: *m/z* (rel. int.) = 212 (25), $C_{15}H_{32}$ [M$^+$], 183 (5), 169 (6), 155 (7), 141 (10), 127 (12), 113 (15), 99 (19), 85 (73), 71 (98), 57 (100). – ^{13}C NMR☂: δ = 31.9, 29.8*, 29.7, 29.4, 22.7**, 14.1***.

n-Hexadecane (**2**): GC-EIMS: *m/z* (rel. int.) = 226 (24), $C_{16}H_{34}$ [M$^+$], 211 (3), 197 (3), 183 (5), 169 (6), 155 (8), 141 (9), 127 (10), 113 (11), 99 (19), 85 (70), 71 (98), 57 (100). – ^{13}C NMR: δ = 31.9, 29.8*, 29.7, 29.4, 22.7**, 14.1***.

n-Heptadecane (**3**): GC-EIMS: *m/z* (rel. int.) = 240 (80), $C_{17}H_{36}$ [M$^+$], 225 (5), 211 (9), 197 (12), 183 (17), 169 (18), 155 (18), 141 (20), 127 (20), 113 (26), 99 (31), 85 (90), 71 (100), 57 (83). – ^{13}C NMR: δ = 31.9, 29.8*, 29.4, 22.7**, 14.1***.

n-Octadecane (**4**): GC-EIMS: *m/z* (rel. int.) = 254 (18), $C_{18}H_{38}$ [M$^+$], 239 (4), 225 (3), 211 (4), 197 (4), 183 (5), 169 (6), 155 (6), 141 (7), 127 (9), 113 (13), 99 (18), 85 (66), 71 (95), 57 (100). – ^{13}C NMR: δ = 31.9, 29.8*, 29.4, 22.7**, 14.1***.

n-Nonadecane (**5**): GC-EIMS: *m/z* (rel. int.) = 268 (20), $C_{19}H_{40}$ [M$^+$], 253 (3), 239 (4), 225 (4), 211 (6), 197 (6), 183 (6), 169 (7), 155 (7), 141 (10), 127 (12), 113 (15), 99 (25), 85 (77), 71 (100), 57 (97). – ^{13}C NMR: δ = 31.9, 29.8*, 29.4, 22.7**, 14.1***.

n-Eicosane (**6**): GC-EIMS: *m/z* (rel. int.) = 282 (12), $C_{20}H_{42}$ [M$^+$], 267 (12), 253 (3), 239 (6), 225 (5), 211 (5), 197 (5), 183 (6), 169 (6), 155 (6), 141 (7), 127 (10), 113 (15), 99 (19), 97 (23), 85 (62), 71 (82), 57 (100). – ^{13}C NMR: δ = 31.9, 29.8*, 29.4, 22.7**, 14.1***.

n-Heneicosane (**7**): GC-EIMS: *m/z* (rel. int.) = 296 (12), $C_{21}H_{44}$ [M$^+$], 281 (3), 267 (3), 253 (3), 239 (4), 225 (4), 211 (4), 197 (4), 183 (5), 169 (5), 155 (6), 141 (7), 127 (10), 113 (12), 111 (16), 99 (17), 97 (26), 85 (62), 71 (90), 57 (100).

n-Docosane (**8**): GC-EIMS: *m/z* (rel. int.) = 310 (82), $C_{22}H_{46}$ [M$^+$], 281 (9), 267 (10), 253 (14), 239 (15), 225 (15), 211 (15), 197 (16), 183 (15), 169 (18), 155 (17), 141 (18), 127 (21), 113 (25), 111 (32), 99 (32), 97 (47), 85 (85), 71 (100), 57 (86). – ^{13}C NMR: δ = 32.1, 29.8*, 29.5, 22.8, 14.1***.

n-Tricosane (**9**): GC-EIMS: *m/z* (rel. int.) = 324 (9), $C_{23}H_{48}$ [M$^+$], 309 (8), 295 (5), 281 (6), 267 (6), 253 (5), 239 (6), 225 (6), 211 (6), 197 (5), 183 (6), 169 (7), 155 (7), 141 (8), 127 (11), 125 (16), 113 (15), 111 (27), 99 (20), 97 (40), 85 (58), 71 (85), 57 (100). – ^{13}C NMR: δ = 32.6, 30.4, 30.0, 23.3, 14.1***.

n-Tetracosane (**10**): GC-EIMS: *m/z* (rel. int.) = 338 (80), $C_{24}H_{50}$ [M$^+$], 309 (10), 295 (12), 281 (14), 267 (16), 253 (15), 239 (15), 225 (16), 211 (15), 197 (15), 183 (16), 169 (15), 155 (16), 141 (17), 127 (18), 125 (23), 113 (22), 111 (41), 99 (28), 97 (58), 85 (72), 71 (100), 57 (85). – ^{13}C NMR: δ = 32.8, 30.6, 30.3, 23.6, 14.1***.

n-Pentacosane (**11**): GC-EIMS: *m/z* (rel. int.) = 352 (75), $C_{25}H_{52}$ [M$^+$], 323 (7), 309 (8), 295 (15), 281 (13), 267 (13), 253 (13), 239 (16), 225 (14), 211 (15), 197 (14), 183 (16), 169 (16), 155 (17), 141 (20), 127 (20), 113 (26), 111 (32), 99 (32), 97 (50), 85 (85), 71 (100), 57 (98).

n-Hexacosane (**12**): GC-EIMS: *m/z* (rel. int.) = 366 (36), $C_{26}H_{54}$ [M$^+$], 337 (5), 323 (5), 309 (7), 295 (7), 281 (8), 267 (8), 253 (8), 239 (9), 225 (8), 211 (9), 197 (9), 183 (10), 169 (10), 155 (12), 141 (13), 127 (15), 113 (18), 111 (24), 99 (27), 97 (35), 85 (77), 71 (100), 57 (97). – ^{13}C NMR: δ = 32.0, 29.7, 29.4, 22.7**, 14.1***.

n-Heptacosane (**13**): GC-EIMS: *m/z* (rel. int.) = 380 (19), $C_{27}H_{56}$ [M$^+$], 365 (5), 351 (4), 337 (4), 323 (5), 309 (4), 295 (4), 281 (6), 267 (5), 253 (6), 239 (6), 225 (6), 211 (6), 197 (6), 183 (6), 169 (7), 155 (8), 141 (9), 127 (12), 113 (17), 111 (18), 99 (22), 97 (27), 85 (71), 71 (95), 57 (100).

n-Octacosane (**14**): GC-EIMS: *m/z* (rel. int.) = 394 (18), $C_{28}H_{58}$ [M$^+$], 379 (6), 365 (4), 337 (6), 323 (5), 309 (5), 295 (5), 281 (6), 267 (5), 253 (5), 239 (7), 225 (6), 211 (7), 197 (8), 183 (6), 169 (8), 155 (7), 141 (9), 127 (12), 113 (15), 111 (25), 99 (26), 97 (39), 85 (72), 71 (90), 57 (100). – ^{13}C NMR: δ = 32.5, 30.3, 29.9, 23.3, 14.1***.

n-Nonadecane (**15**): GC-EIMS: *m/z* (rel. int.) = 408 (27), $C_{29}H_{60}$ [M$^+$], 393 (3), 379 (2), 365 (6), 351 (4), 337 (4), 323 (4), 309 (5), 295 (5), 281 (5), 267 (6), 253 (6), 239 (7), 225 (6), 211 (7), 197 (7), 183 (7), 169 (7), 155 (8), 141 (10), 127 (13), 113 (16), 99 (24), 97 (25), 85 (75), 71 (97), 57 (100).

n-Hentriacontane (**16**): GC-EIMS: *m/z* (rel. int.) = 436 (35), $C_{31}H_{64}$ [M$^+$], 421 (5), 393 (5), 379 (6), 365 (6), 351 (7), 337 (7), 323 (6), 309 (6), 295 (6),

☂ Intensities of peaks observed in ^{13}C NMR spectrum of fraction 'A' are in order * > ** > ***.

281 (7), 267 (6), 253 (6), 239 (9), 225 (7), 211 (9), 197 (8), 183 (10), 169 (8), 155 (10), 141 (13), 127 (15), 113 (20), 99 (27), 97 (35), 85 (80), 71 (95), 57 (100).

Physical and spectral data of fraction 'B'

Yellowish brown thick viscous liquid (2.5 g). UV (CH_3OH) λ_{max} = 356, 277, 224 nm. – FTIR ($CHCl_3$) ν_{max} = 3461 (O-H), 2925 (aromatic and/or vinylic C-H), 2854 (aliphatic C-H), 1770–1680 br. centering at 1738 (various C=O), 1464 and 1378 with shoulder (geminal methyls), 1273 (*t*-butyl), 1163, 1120, 1074 and 1037 (various C-O), 968 (OCH_3), 825, 742, 723 (aromatic fingerprints) cm^{-1}.

Characterization of constituents

2,6-Bis(1,1-dimethylethyl)-4-methyl phenol (**17**): GC-EIMS: *m/z* (rel. int.) = 220 (27), $C_{15}H_{24}O$ [M^+], 205 (100), 189 (5), 177 (7), 161 (7), 145 (10), 119 (7), 109 (11), 105 (10), 91 (20), 81 (11), 69 (30), 57 (18). – ^{13}C NMR: δ = 128.0, 125.5, 34.8, 30.3, 22.6.

3,4-Dihydro-4,4,5,8-tetramethylcoumarin (**18**): GC-EIMS: *m/z* (rel. int.) = 204 (30), $C_{13}H_{16}O_2$ [M^+], 189 (100), 161 (6), 149 (80), 131 (22), 121 (15), 105 (12), 91 (53), 77 (16), 67 (27).

3,4-Dihydro-4,4,7,8-tetramethylcoumarin-6-ol (**19**): GC-EIMS: *m/z* (rel. int.) = 220 (20), $C_{13}H_{16}O_3$ [M^+], 205 (52), 178 (100), 163 (10), 161 (12), 149 (20), 135 (22), 91 (22), 77 (15), 65 (12).

2-(Phenylmethylene)-octanal or α-hexylcinnamaldehyde (**20**): GC-EIMS: *m/z* (rel. int.) = 216 (90), $C_{15}H_{20}O$ [M^+], 185 (5), 173 (25), 159 (20), 145 (58), 141 (15), 131 (40), 129 (100), 117 (75), 115 (50), 104 (12), 91 (66), 82 (12), 77 (10). – ^{13}C NMR: δ = 195.7, 129.7▾, 128.8, 31.5, 29.6, 28.2, 24.8, 22.6, 14.1.

Methyl-3,7,11-trimethyl-2E,6E,10-dodecatrienoate or methyl (2E,6E)-farnesoate (**21**): GC-EIMS: *m/z* (rel. int.) = 250 (5), $C_{16}H_{26}O_2$ [M^+], 234 (12), 219 (45), 207 (7), 191 (8), 173 (10), 145 (30), 136 (7), 129 (45), 117 (40), 114 (30), 105 (8), 95 (8), 91 (42), 81 (22), 69 (100). – ^{13}C NMR δ = 167.7, 130.9, 123.4, 39.7, 26.7, 26.0, 25.7, 15.9.

▾ Broad intense peak.

1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran or galoxolide (**22**): GC-EIMS: *m/z* (rel. int.) = 258 (28), $C_{18}H_{26}O$ [M^+], 243 (100), 228 (5), 213 (18), 198 (3), 185 (5), 171 (5), 155 (3), 171 (3), 69 (4).

Methyl 14-methyl-pentadecanoate (**23**): GC-EIMS: *m/z* (rel. int.) = 270 (22), $C_{17}H_{34}O_2$ [M^+], 239 (8), 227 (13), 213 (3), 199 (4), 185 (6), 171 (5), 157 (3), 143 (20), 129 (7), 111 (3), 97 (7), 87 (71), 74 (100), 69 (15).

Ethyl hexadecanoate or ethyl palmitate (**24**): GC-EIMS: *m/z* (rel. int.) = 284 (14), $C_{18}H_{36}O_2$ [M^+], 239 (9), 213 (3), 199 (4), 185 (4), 157 (14), 143 (6), 115 (6), 101 (55), 88 (100), 73 (13), 61 (9).

Ethyl 9Z-octadecenoate or ethyl oleate (**25**): GC-EIMS *m/z* (rel. int.) = 310 (8), $C_{20}H_{38}O_2$ [M^+], 264 (27), 250 (4), 222 (18), 211 (4), 180 (14), 155 (10), 149 (12), 137 (13), 135 (15), 123 (20), 108 (32), 101 (52), 97 (50), 95 (75), 88 (70), 83 (80), 69 (95), 55 (100). – ^{13}C NMR: δ = 173.7, 130.0, 129.7▾, 34.4, 31.9, 30.0, 29.7, 29.5, 29.3, 29.2, 29.1, 28.9, 28.5, 27.2, 24.9, 22.7, 14.1.

Physical and spectral data of fraction 'C'

Reddish brown thick gummy residue (6.5 g). UV (CH_3OH) λ_{max} = 390, 283, 226 nm. – FTIR ($CHCl_3$) ν_{max} = 3460 br. (O-H), 2928 (aromatic or vinylic C-H), 2856 (aliphatic C-H), 1733, 1669 (various C=O), 1502, 1456 and 1381 (geminal methyls), 1246, 1159, 1029 (various C-O), 956 (OCH_3), 874, 825, 756, 667, 601 (aromatic fingerprints) cm^{-1}.

Characterization of constituents

Methyl 14-methyl pentadecanoate (**23**): GC-EIMS: *m/z* (rel. int.) = 270 (28), $C_{17}H_{34}O_2$ [M^+], 239 (11), 227 (13), 213 (4), 199 (5), 185 (5), 171 (5), 157 (4), 143 (16), 129 (9), 111 (5), 97 (10), 87 (72), 74 (100), 69 (14).

Ethyl hexadecanoate or ethyl palmitate (**24**): GC-EIMS: m/z (rel. int.) = 284 (12), $C_{18}H_{36}O_2$ [M^+], 239 (8), 213 (10), 199 (5), 185 (6), 157 (12), 143 (8), 129 (12), 115 (8), 101 (46), 88 (100), 73 (40), 61 (21).

Ethyl 9Z-octadecenoate or ethyl oleate (**25**): GC-EIMS: *m/z* (rel. int.) = 310 (20), $C_{20}H_{38}O_2$ [M^+], 264 (50), 222 (25), 211 (10), 197 (9), 180 (21), 155 (14), 149 (20), 137 (18), 123 (26), 111 (40), 108 (25), 97 (76), 83 (100), 69 (80). – ^{13}C NMR: δ =

173.7, 130.0, 129.7, 34.4, 31.9, 29.7, 29.5, 29.3, 29.2, 29.1, 28.9, 27.3, 24.8, 22.7, 14.1.

3,7-Dimethyl-1-octen-7-ol or dihydromyrcenol (**26**): GC-EIMS: *m/z* (rel. int.) = 123 [M^+-CH_3-H_2O], 109 (4), 98 (4), 95 (7), 83 (15), 82 (13), 69 (10), 67 (10), 59 (100), 55 (15). – ^{13}C NMR: δ = 44.1, 29.3, 21.9.

1,2,4-Trimethoxy-5-(1Z-propenyl)-benzene or β-asarone (**27**): GC-EIMS: *m/z* (rel. int.) = 208 (100), $C_{12}H_{16}O_3$ [M^+], 193 (35), 177 (4), 165 (20), 162 (10), 150 (5), 137 (5), 119 (4), 105 (5), 91 (8), 77 (5), 69 (10). – ^{13}C NMR: δ = 141.6, 125.4, 124.5, 118.0, 56.8, 55.1.

Pesticidal activity

Raring: The 4th instar larvae of *Anopheles stephensi* Liston (Orangi Town Wild Strain), a vector of the malarial parasite, were collected directly from the natural environment, especially established for this research work. The size of this pond was 8 × 4 feet with a depth of 2 feet. The pupae from the pond were collected and kept in cages for hatching.

Biological test (screening procedure): Ten young 4th instar larvae of *An. stephensi* were collected in 5 ml of tap water and transferred in a glass beaker of 100 ml, containing 45 ml of distilled water. The fractions were tested at 28 ± 1 °C at five final concentrations. The controls were also set. Each concentration and control was run as duplicate set and mortality was recorded after 24 h.

Accurate tests: The WHO method (WHO, 1970) was modified for the application. A batch of 10 insects (4th instar larvae) was released in 100 ml

Table I . Qualitative and quantitative analysis of fraction 'A'.

Compound	GC-FID[a]		GC-EIMS[b]		Identification[e]
	%	R. T.[c]	R. T.[c]	R. T.[d]	
n-Pentadecane (**1**)	0.19	34.62	[f]	11.58	GC-EIMS, GC,[g] ^{13}C NMR
n-Hexadecane (**2**)	0.25	38.82	[f]	13.14	GC-EIMS, GC,[g] ^{13}C NMR
n-Heptadecane (**3**)	0.63	42.91	45.00	14.24	GC-EIMS, GC,[g] ^{13}C NMR
n-Octadecane (**4**)	3.03	46.69	48.28	15.34	GC-EIMS, GC,[g] ^{13}C NMR
n-Nonadecane (**5**)	6.24	50.43	52.00	16.39	GC-EIMS, GC,[g] ^{13}C NMR
n-Eicosane (**6**)	15.77	54.03	55.31	17.44	GC-EIMS, GC,[g] ^{13}C NMR
n-Heneicosane (**7**)	7.82	57.88	58.43	18.43	GC-EIMS, GC,[g]
n-Docosane (**8**)	10.55	62.76	61.55	19.37	GC-EIMS, GC,[g] ^{13}C NMR
n-Tricosane (**9**)	6.43	68.84	65.02	20.37	GC-EIMS, GC,[g] ^{13}C NMR
n-Tetracosane (**10**)	2.65	77.58	68.52	21.25	GC-EIMS, GC,[g] ^{13}C NMR
n-Pentacosane (**11**)	2.72	89.19	[f]	22.25	GC-EIMS
n-Hexacosane (**12**)	4.14[h]	[h]	80.14	23.35	GC-EIMS, ^{13}C NMR
n-Heptacosane (**13**)	0.72[h]	[h]	88.42	25.01	GC-EIMS
n-Octacosane (**14**)	1.80[h]	[h]	[f]	26.44	GC-EIMS, ^{13}C NMR
n-Nonacosane (**15**)	0.30[h]	[h]	[f]	28.53	GC-EIMS
n-Hentriacontane (**16**)	0.17[h]	[h]	[f]	34.55	GC-EIMS

[a] SPB-5®; Supelco capillary column containing 5% phenyl- and 95% methyl silicone as stationary phase.
[b] HP-5®; Hewlett-Packard capillary column chemically equivalent to SPB-5®.
[c,d] GC oven cycle (program A and B, respectively, *vide* Experimental).
[e] Further supported by UV and FTIR spectroscopy of fraction 'A'.
[f] MS not obtained in that particular program.
[g] Identifications made by using standards (co-injection).
[h] Poor broad peaks in program A, corresponding percentages are calculated from program B.

beaker, containing 50 ml filtered tap water. The concentrations selected in the preliminary screening of each compound were tested at 28 ± 1 °C. A group of 7 beakers was set up, five for different concentrations and one each for control and check. Each experiment was repeated five times. The experiment was discarded if the mortality was found more than 10% in control. The mortality was recorded after 24 h and readings were subjected to Abbot's formula (Abbot, 1925).

Calculations of LC_{50}: The lethal concentrations (LC_{50}) were calculated using PROBIT analysis (Raymond *et al.*, 1993).

Results and Discussion

The fruit coating extract was found more active than the seed extract (Tariq *et al.*, 2001, 2002), therefore, the non-polar to less polar fractions 'A', 'B' and 'C', obtained after partial purification through VLC from the fruit coating extract (Siddiqui *et al.*, 2000a, 2002), were subjected to GC-FID and GC-EIMS analysis (Masada, 1976) and the components of these fractions were characterized mainly by mass spectral survey (NIST Mass Spectral Search Progam, 1998; GC-MS Library of Shimadzu, 1996). This resulted in the identification of the constituents in the fractions which were further supported with Kovats retention indices (RI) cited in the literature (Kovats, 1958; Davies, 1990).

Comparison of the ^{13}C NMR spectra of the mixture with those recorded for the pure authentic compounds in literature (Crombie *et al.*, 1975; Patra and Mitra, 1981; Kubeczka and Formacek, 1982; Clayden *et al.*, 2001; Pouchert and Behnke, 1992) and tentative interpretation of UV and IR spectra further helped in the identification. The absorbance and chemical shift values obtained for the compounds in the fractions were in good agreement with the reported data.

Ethyl esters of fatty acid **23** and **24** were prepared and injected to verify their presence in the mixture. Some other compounds were also iden-

Table II. Quantitative and qualitative analysis of fraction 'B' and 'C'.

Compound	GC-FID[a]		GC-EIMS[b]	Identification[d]
	%	RI[c]	RI[c]	
2,6-*Bis*-(1,1-dimethylethyl)-4-methyl phenol (**17**)	3.11	1493	1500	GC-EIMS ^{13}C NMR[e]
3,4-Dihydro-4,4,5,8-tetramethylcoumarin (**18**)	1.08	1519	1534	GC-EIMS
3,4-Dihydro-4,4,7,8-tetramethylcoumarin-6-ol (**19**)	0.58	1551	1542	GC-EIMS
α-Hexylcinnamaldehyde (**20**)	8.57	1765	1732	GC-EIMS ^{13}C NMR[e]
Methyl (2*E*,6*E*)-farnesoate (**21**)	5.71	1789	1786	GC-EIMS, ^{13}C NMR[e]
Galoxolide (**22**)	2.60	1838	1828	GC-EIMS
Methyl 14-methyl-pentadecanoate (**23**)	1.14 2.29[f]	1945 1929[f]	1908 1900[f]	GC-EIMS
Ethyl palmitate (**24**)	5.05 3.78[f]	1979 1971[f]	1990 1992[f]	GC-EIMS, RI,[g] GC,[h]
Ethyl oleate (**25**)	2.32 1.96[f]	2178 2177[f]	2171 2161[f]	GC-EIMS, GC,[h] ^{13}C NMR[e]
Dihydromyrcenol (**26**)	20.12[f]	1068[f]	1070[f]	GC-EIMS, RI,[g] ^{13}C NMR[e]
β-Asarone (**27**)	2.17[f]	1628[f]	1634[f]	GC-EIMS, ^{13}C NMR[e]

[a] SPB-5®; Supelco capillary column containing 5% phenyl- and 95% methyl silicone as stationary phase.
[b] HP-5®; Hewlett-Packard capillary column chemically equivalent to SPB-5®.
[c] GC oven cycle (program A, *vide* Experimental).
[d] Further supported by UV and FTIR spectroscopy of fractions.
[e] Pouchert and Behnke, 1992; Clayden *et al.*, 2001; Crombie *et al.*, 1975; Patra and Mitra, 1981.
[f] Belongs to fraction 'C'.
[g] Davies, 1990.
[h] Identifications made by using standards (co-injection).

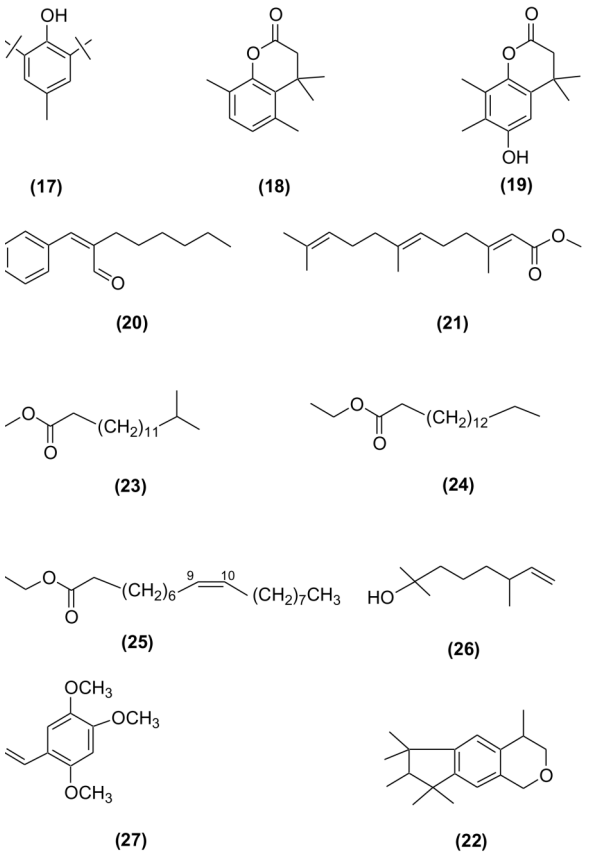

Fig. 1. Components of fraction 'B' and 'C'; 2,6-*bis*-(1,1)-dimethylethyl-4-methyl phenol (**17**), 3,4-dihydro-4,4,5,8-tetramethylcoumarin (**18**), 3,4-dihydro,4,4,7,8-tetramethylcoumarin-6-ol (**19**), α-hexylcinnamaldehyde (**20**), methyl (2*E*,6*E*)-farnesoate (**21**), galoxolide (**22**), methyl 14-methyl-pentadecanoate (**23**), ethyl palmitate (**24**), ethyl oleate (**25**), dihydromyrcenol (**26**), β-asarone (**27**).

tified by co-injection. Table I and II show the results of qualitative and quantitative analysis of these fractions.

Fraction 'A' showing an LC_{50} of 100 ppm was found to contain hydrocarbons. The UV spectrum was transparent up to the solvent cut-off. ν_{max} in the FTIR spectrum was comparable to that of white paraffin oil, which is also a mixture of *n*-alkanes. The ^{13}C NMR showed no resonance except those specifics to hydrocarbons (Pouchert and Behnke, 1992). Thus sixteen hydrocarbons **1–16**, were identified from fraction 'A' (Table I). All hydrocarbons except **6** and **8** were identified for the first time in the fruit coatings. However, hydrocarbons **4**, **5**, **8** and **11** to **16** were also been reported from the leaves and blossoms of the plant (Siddiqui *et al.*, 1988, 1992; Akhila and Rani, 1999). The use of hydrocarbons as a common domestic pesticide is well established (*loc. cit.*).

Yellowish brown thick viscous liquid of fraction 'B' (2.5 g), showing pesticidal activity LC_{50} 200 ppm, displayed broad obscured maxima in UV spectrum centering at 224, 277 and 356 nm. These absorptions indicated benzenoid systems with bathochromic shifts as compared to the maxima of benzene itself (Mendham *et al.*, 2000). The ^{13}C NMR spectrum of fraction 'B' was also compared with the ^{13}C NMR spectra of pure compounds and several peaks of pure compounds were identified in the ^{13}C NMR spectrum of fraction 'B' (Table II).

Altogether nine compounds (Fig. 1) were identified from fraction 'B' including two aromatics 2,6-*bis*(1,1-dimethylethyl)-4-methylphenol (**17**, 3.11%) and 2-(phenylmethylene)-octanal or α-hexylcinnamaldehyde (**20**, 8.57%), three benzopyranoids 3,4-dihydro-4,4,5,8-tetramethylcoumarin (**18**, 1.08%), 3,4-dihydro-4,4,7,8-tetramethylcoumarin-6-ol (**19**, 0.58%) and 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[*g*]-2-benzopyran or galoxolide (**22**, 2.60%), and one sesquiterpene methyl-3,7,11-trimethyl-2*E*,6*E*,10-dodecatrienoate or methyl (2*E*,6*E*)-farnesoate (**21**, 5.71%). Three esters of fatty acids were also identified. These include the methyl 14-methyl-pentadecanoate (**23**, 1.14%), ethyl hexadecanoate or ethyl palmitate (**24**, 5.05%) and ethyl 9*Z*-octadecenoate or ethyl oleate (**25**, 2.32%; Table II). Except **24** and **25** all these compounds were identified for the first time in the plant, although several esters of fatty acids are reported from the oil and seeds of the plant (Siddiqui *et al.*, 1988; Ali *et al.*, 1996; Kaushik and Vir, 2000; Akhila and Rani, 1999). **17** is a reputed antioxidant and a report on the antioxidant activity of the neem has also appeared (Rao *et al.*, 1998). Neem is a rich source of triterpenoids but **23** is the first sesquiterpene reported from neem.

Fraction 'C' (6.5 g) was obtained as reddish brown gummy residue that showed pesticidal activity with LC_{50} 150 ppm. The λ_{max} in UV spectrum were observed at 226, 283 and 390 nm indicating benzenoid system shifted towards higher wavelength (Mendham *et al.*, 2000). A total of five compounds was identified in this fraction. Three were the same esters of fatty acids, **25–27**, as identified in fraction 'B' (Table II) although their concentrations were different. The remaining two compounds included a monoterpene 3,7-

dimethyl-1-octen-7-ol or dihydromyrcenol (**28**; 20.12%) and an aromatic constituent 1,2,4-trimethoxy-5-(1*Z*-propenyl)-benzene or β-asarone (**29**; 2.17%). Compound **28** is the first monoterpene reported from neem. The ^{13}C NMR and FTIR spectra of fraction 'C' were interpreted as discussed for the fraction 'B' (Table II).

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