

印楝提取物的杀虫活性及其中四降三萜研究*

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摘要 从印楝(*Azadirachta indica*)种仁的提取物中分离得到10个四降三萜化合物,通过波谱方法鉴定,它们分别是 nimbin(1), deacetylnimbin(2), salannolide(3), azadirachtin(4), vepaol(5), isovepaol(6), 3-tigloyl-azadirachtol(7), 1-tigloyl-3-acetyl-azadirachtinin(8), 3-tigloyl-azadirachtinin(9), 1-tigloyl-3-acetyl-11-hydroxymeliacarpinin(10)。此外,还得到β-谷甾醇、豆甾醇、胡萝卜甙和硬脂酸。含印楝素(azadirachtin)为0.5%的印楝提取物在稀释150~800倍时对小菜蛾和菜粉蝶有较强的防治作用。

关键词 印楝; 楝科; 四降三萜; 杀虫作用

印楝(*Azadirachta indica*)为楝科(Meliaceae)植物,该植物富含印楝素(azadirachtin)等四降三萜杀虫活性成分,具有很强的杀虫、拒食、抑制害虫生长、延缓昆虫产卵、抑制昆虫激素分泌、降低昆虫生育能力等生理活性;此外,还有广泛的药用价值,如抗疟疾、退热、驱虫、抑制动物和人体内的真菌生长、抗生育力等^[1]。印度、缅甸、非洲等地区都有栽种。我们从缅甸采集的印楝种仁的甲醇提取物中分离到10个四降三萜,由各项波谱分析和文献报道数据比较分别鉴定为 nimbin(1)^[2], deacetylnimbin(2)^[3], salannolide(3)^[4], azadirachtin(4)^[5], vepaol(5)^[5], isovepaol(6)^[6], 3-tigloyl-azadirachtol(7)^[5], 1-tigloyl-3-acetyl-

azadirachtinin(8)^[7], 3-tigloyl-azadirachtinin(9)^[8], 1-tigloyl-3-acetyl-11-hydroxymeliacarpinin(10)^[9]。含0.5%印楝素(azadirachtin)的乳剂在稀释150~800倍对小菜蛾和菜粉蝶有较强的拒食作用,减少取食量,致使幼虫延迟蜕皮,蜕皮畸形至死亡,各处理浓度15~20 d 小菜蛾幼虫死亡率60%~90%;菜粉蝶幼虫死亡率96%~100%。0.5%印楝素(azadirachtin)的乳剂对小菜蛾和菜粉蝶幼虫处理后影响化蛹率和羽化率,不仅产卵大大减少,且所产卵不能孵化。0.5%印楝素乳剂对小菜蛾及菜粉蝶三龄幼虫的室内毒力测定结果见表1和表2。

表1 0.5%印楝素乳剂对小菜蛾三龄幼虫的室内毒力测定(20℃)

Table 1 The results of neem extracts containing 0.5% azadirachtin against *Plutella xylostella* L.

浓度(X) Concentration	处理方式 Treatment way	供试虫数 The number of <i>Plutella xylostella</i> L.	幼虫死亡率(%) Mortality of larva				化蛹率(%) Rate of pupation	羽化率(%) Rate of eclosion	产卵(粒/头) Rate of oviposition	孵化率(%) Rate of hatching
			3 d	7 d	10 d	15 d				
800X	浸叶 Leaf soaking	250	5.6	22.8	58.4	68.0	32.0	33.7	4.6	0
600X	浸叶 Leaf soaking	250	6.0	30.0	76.0	80.4	19.6	10.2	10.0	0
400X	浸叶 Leaf soaking	250	7.2	21.6	73.2	88.8	11.2	10.7	0	0
300X	浸叶 Leaf soaking	250	7.6	30.0	76.0	97.6	2.4	0	0	0
150X	浸叶 Leaf soaking	250	17.2	36.4	84.4	98.0	2.0	0	0	0
800X	喷雾 Spray	250	23.6	38.0	53.2	66.8	33.2	20.5	2.3	0
600X	喷雾 Spray	250	25.2	43.2	61.6	82.0	18.0	15.6	0	0
400X	喷雾 Spray	250	29.2	45.6	63.2	94.0	6.0	6.7	0	0
300X	喷雾 Spray	250	36.8	54.8	72.0	97.6	2.4	0	0	0
150X	喷雾 Spray	250	29.6	59.6	76.8	99.6	0.4	0	0	0
CK		250	1.6	5.6	—	—	94.4	100	99.4	94.8

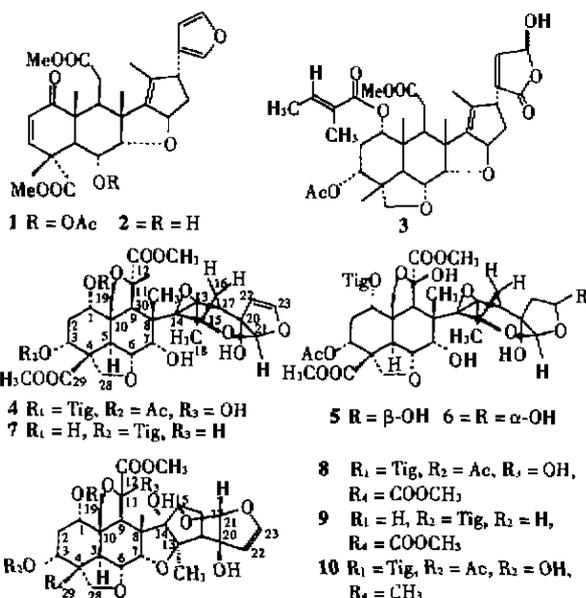
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表2 0.5%印楝素乳剂对菜粉蝶三龄幼虫的室内毒力测定(20℃)

Table 2 The results of neem extracts containing 0.5% azadirachtin against *Piers rapae* L.

浓度(X) Concentration	处理方式 Treatment way	供试虫数 The number of <i>Plutella zylotilla</i> L.	幼虫死亡率(%) Mortality of larva					化蛹率(%) Rate of pupation	羽化率(%) Rate of eclosion
			3 d	7 d	10 d	15 d	20 d		
800X	浸叶 Leaf soaking	100	4.0	22.0	40.0	96.0	—	4.0	0
600X	浸叶 Leaf soaking	100	6.0	34.0	42.0	86.0	100	0	0
400X	浸叶 Leaf soaking	100	6.0	24.0	50.0	96.0	100	0	0
300X	浸叶 Leaf soaking	100	40.0	72.0	100	—	—	0	0
150X	浸叶 Leaf soaking	100	18.0	50.0	86.0	100	—	—	0
800X	喷雾 Spray	100	2.0	10.0	50.0	96.0	—	4.0	0
600X	喷雾 Spray	100	4.0	12.0	58.0	98.0	—	2.0	0
400X	喷雾 Spray	100	10.0	38.0	82.0	100	—	0	0
300X	喷雾 Spray	100	28.0	60.0	84.0	100	—	0	0
150X	喷雾 Spray	100	2.0	46.0	84.0	100	—	0	0
CK		100	0	0	2.0	4.0	—	96.0	91.5



1 实验部分

1.1 仪器与材料

熔点用四川大学仪器厂 XRC-1 显微熔点测定仪测定, 温度未经校正; IR 光谱用 Bio-Rad 135 型分光光度计测定, KBr 压片; UV 光谱使用日本岛津 UV-210A 仪测定; MS 用 VG Autospec-3000 质谱仪测定, EIMS: 70 eV; NMR 用 Bruker AM-400 超导核磁共振仪测定, 以 TMS 为作内标。各种层析用硅胶青岛海洋化工厂出品。印楝种子采于缅甸曼德勒地区。

小菜蛾 (*Plutella zylotilla* L.) 为室内饲养的敏感

品系, 菜粉蝶 (*Piers rapae* L.) 为田间采集的幼虫, 都以三龄幼虫供试。叶片浸渍法为将甘蓝叶洗净凉干后, 在供试药液内浸渍 1 分钟, 凉干后接虫供试。喷雾法为将幼虫接入洗净凉干的甘蓝叶后, 以手提喷雾器均匀喷雾, 使虫体和叶片均匀接触供试药液。

1.2 提取与分离

经风干粉碎的 1.36 kg 印楝种仁, 以石油醚脱脂 3 次, 脱脂种仁用甲醇回流提取 4 次, 得甲醇提了物。提取物依次用石油醚、乙酸乙酯萃取各三次, 回收溶剂, 乙酸乙酯萃取物部分 (37 g) 经硅胶柱层析得化合物 (1) (178 mg, 得率 0.013%), (2) (133 mg, 得率 0.009%), (3) (78 mg, 得率 0.0057%), (4) (2.5 g, 得率 0.183%), (5) (30 mg, 得率 0.0022%), (6) (16 mg, 得率 0.0012%), (7) (42 mg, 0.0031%), (8) (125 mg, 得率 0.0092%), (9) (40 mg, 得率 0.0029%) 和 (10) (36 mg, 得率 0.0026%)。

2 鉴定

2.1 Nimbin(1)

无色针晶 (Me₂CO); mp. 202 ~ 204 °C; UV (MeOH) λ_{max} (log_e) 211.5 (4.27) nm; IR (KBr) ν_{max} 3100, 1750, 1710, 1625, 1370, 867, 785, 693 cm⁻¹; EIMS m/z 540 [M]⁺ (67), 509 (18), 498 (7), 480 (23), 421 (10), 383 (25), 340 (20), 273 (34), 259 (20), 231 (100), 215 (15), 201 (18), 187 (19), 174 (26), 159 (2), 147 (27), 112 (26), 91 (33), 81 (20), 59 (37); ¹H NMR 数据与文献^[2]报道一致, ¹³C NMR 数据见表 3。

2.2 Deacetylnimbin(2)

无色针晶 (Me₂CO); mp. 208 ~ 210 °C; UV (MeOH) λ_{max} (log ϵ) 211 (4.20) nm; IR (KBr) ν_{max} 3463, 2954, 1734, 1683, 1437, 1259, 1229, 1071, 1031, 1055 cm⁻¹; EIMS *m/z* 498[M]⁺ (88), 480(12), 467(27), 421(8), 389(23), 371(5), 268(35), 231(100), 215(10), 199(15), 187(17), 159(21), 145(25), 119(21), 105(26), 91(34), 81(22), 69(13), 59(26); ¹H NMR 数据与文献^[3]报道一致; ¹³C NMR 数据见表3。

2.3 Salannolide(3)

无色方晶 (Me₂CO); mp. 292 ~ 294 °C; UV (MeOH) λ_{max} (log ϵ) 207 (4.08) nm; IR (KBr) ν_{max} 3399, 2932, 1760, 1735, 1709, 1653, 1439, 1249, 1052, 1008, 952 cm⁻¹; EIMS *m/z* 628[M]⁺ (53), 613(7), 610(8), 545(8), 528(10), 315(30), 235(12), 207

(8), 189(5), 175(5), 147(9), 131(7), 119(12), 105(10), 83(100); ¹H NMR 数据与文献^[4]报道一致; ¹³C NMR 数据见表3。

2.4 Azadirachtin(4)

白色粉末; mp. 155 ~ 157 °C; UV (MeOH) λ_{max} (log ϵ) 217 (4.11) nm; IR (KBr) ν_{max} 3450, 2940, 1730, 1645, 1615, 1370, 1260, 1045, 920, 735 cm⁻¹; EIMS *m/z* 702[M-H₂O]⁺ (3), 688[M-MeOH]⁺ (3), 660[M-HOAc]⁺ (2), 643[M-H₂O-COOMe]⁺ (13), 620(16), 603(12), 559(18), 544(7), 461(5), 409(8), 347(9), 315(5), 291(10), 273(8), 251(10), 233(14), 201(13), 183(17), 151(64), 123(23), 83(100); FAB-MS *m/z* 719[M-H]⁻; ¹H NMR 数据与文献^[5]报道一致; ¹³C NMR 数据见表3。

表3 化合物1~10的¹³C NMR 化学位移值*

Table 3 ¹³C NMR spectral Data for Compounds 1~10(100 MHz)

C	1	2	3	4	5	6	7	8	9	10
1	201.4 s	202.1 s	72.3 d	70.7 d	70.7 d	70.7 d	69.3 d	70.1 d	69.3 d	70.9 d
2	126.0 d	126.4 d	27.6 t	29.8 t	29.7 t	29.8 t	32.0 t	30.1 t	32.7 t	29.6 t
3	147.5 d	148.1 d	72.1 d	67.0 d	67.1 d	67.1 d	67.6 d	67.0 d	68.2 d	70.2 d
4	48.0 s	47.8 s	43.2 s	52.6 s	52.7 s	52.6 s	53.3 s	52.8 s	53.2 s	42.3
5	41.6 d	43.7 d	40.2 d	37.1 d	37.0 d	37.1 d	35.1 d	36.4 d	37.7 d	35.3 d
6	68.7 d	66.2 d	72.9 d	73.9 d	74.0 d	73.9 d	74.3 d	72.0 d	72.4 d	71.3 d
7	84.6 d	87.0 d	86.7 d	74.4 d	74.1 d	74.2 d	73.6 d	82.3 d	80.7 d	82.9 d
8	48.0 s	47.5 s	49.3 s	45.6 s	45.5 s	45.6 s	44.1 s	51.0 s	48.6 s	51.0 s
9	38.7 d	39.1 d	39.8 d	44.8 d	44.8 d	44.8 d	43.8 d	46.6 s	47.4 d	49.2 d
10	47.1 s	47.4 s	40.0 s	50.3 s	50.2 s	50.3 s	51.2 s	50.1 s	51.4 s	49.9 s
11	34.2 t	34.3 t	31.2 t	104.2 s	104.3 s	104.0 s	79.4 d	104.2 s	77.5 d	107.8 s
12	174.5 s	175.5 s	173.6 s	171.8 s	171.8 s	171.8 s	173.4 s	170.9 s	175.4 s	170.9 s
13	135.0 s	134.9 s	132.3 s	68.6 s	68.7 s	68.3 s	66.6 s	94.2 s	92.4 s	94.6 s
14	146.2 s	146.7 s	149.6 s	70.1 s	69.6 s	69.6 s	69.3 s	93.9 s	94.6 s	93.2 s
15	87.1 d	87.6 d	87.7 d	76.5 d	76.8 d	76.7 d	76.1 d	80.6 d	80.4 d	81.0 d
16	41.6 t	41.4 t	40.2 t	25.1 t	24.6 t	24.4 t	25.0 t	29.6 t	29.6 t	29.5 t
17	49.5 d	49.7 d	49.8 d	48.6 d	49.7 d	49.2 d	48.8 d	50.9 d	51.3 d	52.2 d
18	12.8 q	12.8 q	16.8 q	18.4 q	18.3 q	18.4 q	18.4 q	26.4 q	25.2 q	26.4 q
19	16.7 q	17.2 q	14.9 q	69.1 t	69.0 t	69.6 t	71.3 t	70.4 t	70.5 t	70.1 t
20	126.8 s	126.8 s	138.4 s	83.6 s	80.9 s	80.0 s	83.6 s	86.3 s	86.3 s	86.3 s
21	139.0 d	139.0 d	172.3 s	108.8 d	106.7 d	106.7 d	109.0 d	109.2 d	109.2 d	109.1 d
23	143.0 d	43.0 d	98.1 d	147.0 d	106.0 d	104.2 d	146.6 d	145.8 d	145.8 d	145.8 d
28	173.5 s	173.6 s	77.8 t	73.0 t	74.4 t	73.0 t	73.2 t	72.0 t	72.9 t	76.5 t
29	17.2 q	17.5 q	19.5 q	173.4 s	173.4 s	173.4 s	174.0 s	173.0 s	172.7 s	18.3 q
30	16.7 q	16.4 q	13.2 q	21.4 q	21.5 q	21.5 q	21.2 q	17.6 q	15.3 q	17.4 q
COOCH ₃	52.9 q	52.9 q	52.0 q	53.2 q	53.1 q	53.2 q	53.3 q	53.6 q	53.0 q	53.3 q
	51.5 q	51.6 q		52.6 q	52.7 q	52.6 q	52.7 q	52.8 q	53.4 q	
OAc	20.9 q		20.8 q	20.8 q	20.7 q	20.8 q		21.0 q		20.8 q
	17.05 s		170.3 s	169.6 s	169.8 s	169.5 s		169.7 s		170.2 s
tigloyl										
C-1'			166.8 s	166.3 s	166.3 s	166.2 s	167.0 s	166.5 s	166.1 s	166.7 s
C-2'			129.8 s	128.7 s	128.6 s	128.7 s	128.4 s	129.9 s	127.6 s	128.6 s
C-3'			137.3 d	137.6 d	137.7 d	137.6 d	138.6 d	137.4 d	139.2 d	137.8 d
C-4'			14.2 q	14.2 q	14.2 q	14.2 q	14.5 q	14.1 q	14.5 q	14.0 q
C-5'			12.2 q	11.9 q	11.8 q	11.9 q	11.9 q	12.2 q	12.0 q	12.0 q
23-OCH ₃				55.6 q	54.9 q					

* 化合物3在 C₅D₅N 中测定,其余化合物均在 CDCl₃中测定,以 TMS 为内标。

Compound 3 was measured in C₅D₅N, while other compounds in CDCl₃ with TMS as internal standard.

2.5 Vepaol(5)

白色粉末; mp. 140~142 °C; UV (MeOH) λ_{\max} (log ϵ) 215 (4.04), 283 (3.39) nm; IR (KBr) ν_{\max} 3461, 2957, 1740, 1650, 1439, 1378, 1269, 1157, 1042, 938 cm^{-1} ; FAB-MS m/z 751 [M-H]⁻; ¹H NMR 数据与文献^[5]报道一致; ¹³C NMR 数据见表3。

2.6 Isovepaol(6)

白色粉末; mp. 158~160 °C; UV (MeOH) λ_{\max} (log ϵ) 215 (3.94) nm; IR (KBr) ν_{\max} 3448, 2956, 1740, 1710, 1651, 1439, 1379, 1270, 1140, 1045, 957, 928 cm^{-1} ; EIMS m/z 703 [M-H₂O-OCH₃]⁺ (3), 662 (6), 644 (3), 624 (19), 374 (6), 315 (8), 283 (6), 251 (10), 231 (12), 203 (11), 189 (18), 159 (51), 143 (100), 117 (26), 103 (95), 83 (84); FAB-MS m/z 751 [M-H]⁻; ¹H NMR (CDCl₃, 400 MHz) δ 4.72 (1 H, dd, J = 2.8, 2.8 Hz, H-1), 2.26 (1 H, dd, J = 16.5, 3.2 Hz, H-2 α), 2.13 (1 H, dd, J = 16.5, 3.2 Hz, H-2 β), 3.34 (1 H, d, J = 12.5 Hz, H-5), 4.56 (1 H, dd, J = 12.5, 2.6 Hz, H-6), 4.56 (1 H, d, J = 2.5 Hz, H-7), 3.28 (1 H, s, H-9), 4.67 (1 H, d, J = 3.4 Hz, H-15), 1.64 (1 H, m, H-16a), 1.90 (1 H, d, J = 13.0 Hz, H-16b), 2.41 (1 H, d, J = 4.5 Hz, H-17), 2.03 (3 H, s, H-18), 3.64 (1 H, d, J = 9.6 Hz, H-19a), 4.10 (1 H, d, J = 9.6 Hz, H-19b), 5.50 (1 H, s, H-21), 2.22, 2.38 (each 1 H, m, H-22), 5.10 (1 H, d, J = 6.0 Hz, H-23), 4.04 (1 H, d, J = 9.0 Hz, H-28a), 3.72 (1 H, d, J = 9.0 Hz, H-28b), 1.75 (3 H, s, H-30), 3.63 (3 H, s, 12-OCH₃), 3.35 (3 H, s, 23-OCH₃), 3.77 (1 H, s, 29-OCH₃), 1.96 (3 H, s, OAc), 6.89 (1 H, qq, J = 7.0, 1.5 Hz, H-3'), 1.77 (3 H, d, J = 7.0 Hz, H-4'), 1.85 (3 H, s, H-5'); ¹³C NMR 数据见表3。

2.7 3-Tigloyl-azadirachtol(7)

白色粉末; mp. 149~151 °C; UV (MeOH) λ_{\max} (log ϵ) 205 (4.03) nm; IR (KBr) ν_{\max} 3407, 2956, 1729, 1650, 1440, 1383, 1266, 1077, 1038, 977, 939, 755 cm^{-1} ; EIMS m/z 662 [M]⁺ (3), 644 [M-H₂O]⁺ (5), 603 [M-OAc]⁺ (5), 579 [M-83]⁺ (2), 479 [579-C₈H₇O₃]⁺ (12), 449 (50), 367 (8), 344 (5), 289 (15), 273 (17), 243 (12), 219 (17), 195 (22), 167 (16), 149 (40), 124 (34), 105 (41), 83 [C₈H₇O]⁺ (100); FAB-MS m/z 661 [M-H]⁻; ¹H NMR 数据与文献^[5]报道一致; ¹³C NMR 数据见表3。

2.8 1-Tigloyl-3-acetyl-azadirachtinin(8)

白色粉末; mp. 174~176 °C; UV (MeOH) λ_{\max} (log ϵ) 4.17 (4.08) nm; IR (KBr) ν_{\max} 3420, 3400, 2940, 1730, 1645, 1615, 1435, 1260, 1040, 945, 920, 740 cm^{-1} ; EIMS m/z 702 [M-H₂O]⁺ (2), 688 [M-MeOH]⁺ (2), 656 (6), 627 (11), 549 (9), 512 (11), 476 (3), 412 (4), 315 (3), 235 (10), 218 (8), 195 (15), 159 (11), 136 (28), 121 (16), 97 (20), 83 [C₈H₇O]⁺ (100); FAB-MS m/z 719 [M-H]⁻; ¹H NMR 数据与文献^[7]报道一致; ¹³C NMR 数据见表3。

2.9 3-Tigloylazadirachtinin(9)

白色粉末; mp. 131~133 °C; UV (MeOH) λ_{\max} (log ϵ) 211.5 (3.94) nm; IR (KBr) ν_{\max} 3431, 2958, 1740, 1651, 1626, 1439, 1380, 1270, 1040, 982, 950, 925, 884, 783 cm^{-1} ; EIMS m/z 662 [M]⁺ (3), 591 [M-C₃H₃O₂]⁺ (32), 479, 449, 247, 195, 95, 83 [C₈H₇O]⁺ (100); FAB-MS m/z 661 [M-H]⁻; ¹H NMR 数据与文献^[8]报道一致; ¹³C NMR 数据见表3。

2.10 1-Tigloyl-3-acetyl-11-hydroxymellacarpinin(10)

白色粉末; mp. 128~130 °C; UV (MeOH) λ_{\max} (log ϵ) 215 (3.96) nm; IR (KBr) ν_{\max} 3413, 2958, 1740, 1651, 1439, 1380, 1270, 1160, 1040, 982, 950, 925 cm^{-1} ; EIMS m/z 675 [M-H]⁺ (100), 643 (42), 571 (10), 491 (14), 339 (4), 325 (13), 311 (50), 255 (3), 99 (28), 59 (8); ¹H NMR (CDCl₃, 400 MHz) δ 4.61 (1 H, dd, J = 2.8, 3.2 Hz, H-1), 2.18, 2.07 (each 1 H, m, H-2), 4.85 (1 H, dd, J = 3.0, 2.6 Hz, H-3), 2.39 (1 H, d, J = 12.8 Hz, H-5), 3.94 (1 H, dd, J = 12.8, 2.9 Hz, H-6), 4.44 (1 H, d, J = 2.8 Hz, H-7), 3.55 (1 H, brs, H-9), 3.95 (1 H, m, H-15), 1.88, 2.10 (each 1 H, m, H-16), 2.13 (1 H, m, H-17), 1.46 (3 H, s, H-18), 3.76 (1 H, d, J = 9.2 Hz, H-19a), 4.00 (1 H, d, J = 9.2 Hz, H-19b), 5.58 (1 H, s, H-21), 4.83 (1 H, d, J = 2.9 Hz, H-22), 6.32 (1 H, d, J = 2.9 Hz, H-23), 3.47 (1 H, d, J = 8.0 Hz, H-28a), 3.49 (1 H, d, J = 8.0 Hz, H-28b), 0.94 (3 H, s, H-29), 1.57 (3 H, s, H-30), 3.61 (3 H, s, 12-OCH₃), 1.89 (3 H, s, OAc), 6.88 (1 H, qq, J = 7.0, 1.3 Hz, H-3'), 1.79 (3 H, d, J = 6.4 Hz, H-4'), 1.80 (3 H, s, H-5'); ¹³C NMR 数据见表3。

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TETRANORTRITERPENOIDS FROM INSECTICIDAL FRACTION OF AZADIRACHTA INDICA

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Abstract Ten known tetranortriterpenoids, nimbin (1), deacetylnimbin (2), salannolide (3), azadirachtin (4), vepaol (5), isovepaol (6), 3-tigloyl-azadirachtol (7), 1-tigloyl-3-acetyl-azadirachtinin (8), 3-tigloyl-azadirachtinin (9), 1-tigloyl-3-acetyl-11-hydroxymeliacarpinin (10), were isolated from the seed kernels of *Azadirachta indica*. Their structures were identified on the basis of spectral methods. The extracts containing 0.5% azadirachtin and above ten tetranortriterpenoids show significant insecticidal activity to *Plutella xylostella* L. and *Piers rapae* L.

Key words *Azadirachta indica*; Meliaceae; Tetranortriterpenoids; Insecticide

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