CHAPTER - IV

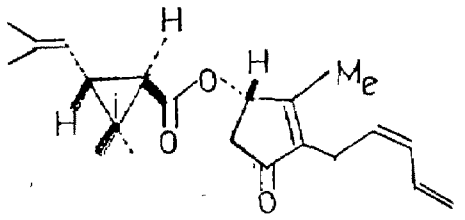
SCREENING OF PLANT MATERIALS FOR INSECT CONTROL

INTRODUCTION

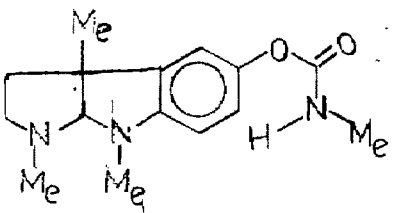
Many plants exhibiting insecticidal properties have been known since time immemorial. It is only during the last 150 years that serious attempts have been made to unreveal the chemical structures of the active principles occuring in many of the well known insecticidal plants. Over the past 50 years, more than 2000 plant species belonging to different families and genera have been reported to contain toxic principles which are effective against many insects.[1] The insecticidal chemicals from natural sources have intrigued, challenged and delighted, chemist for more than a century. Despite the current spurt of activity, a majority of the reported insecticidal plants remain chemically obscure. Much efforts have been put forth to isolate and characterise such plant products and also to standardise desired response through bioassay procedures. The need for natural products has come up due to the aggricultural pests having developed resistance towards a number of synthetic insecticides and such insecticides have inturn also secondary effects on environment leaving residues beyond codex tolerance level. Thus such a situation demands for research in these areas for the development of effective alternative pest control substance having practically no residual toxic effects on the eco-system. Therefore, today we have a number of contributions available in literature, which report many plant isolates of manifold activities i.e. pheromonal compounds,[2] juvenile hormone mimics, insecticides, antifeedant compounds, antigonadal compounds[3] etc.

INSECTICIDES

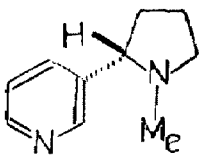
Among the well reputed plants, which still enjoy popularity as insecticides are "Pyrethrum", the dried flowers of Chrysanthemum cinerariaefolium



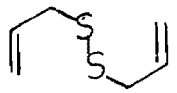
(1) Pyrethrin-1



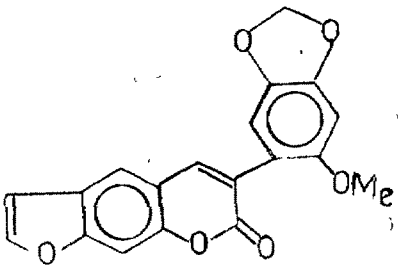
(2) Physostigmine



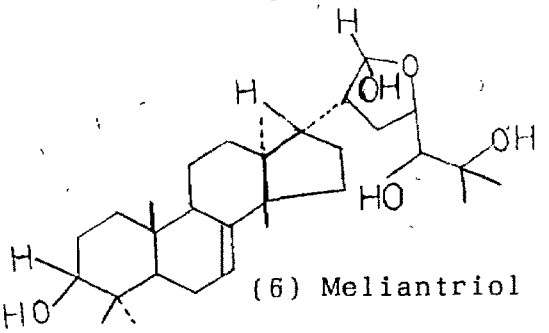
(3) Nicotine



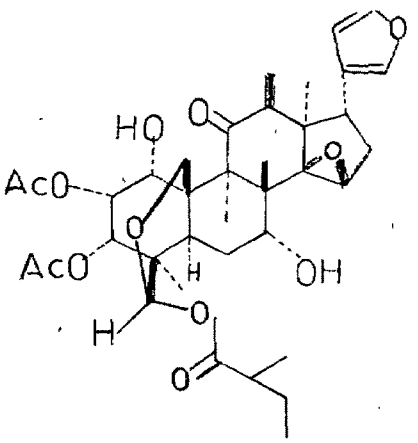
(4) Diallyl disulfide



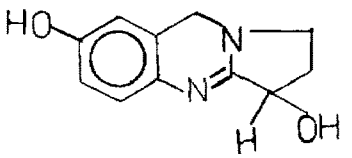
(5) Pachyrrhizin



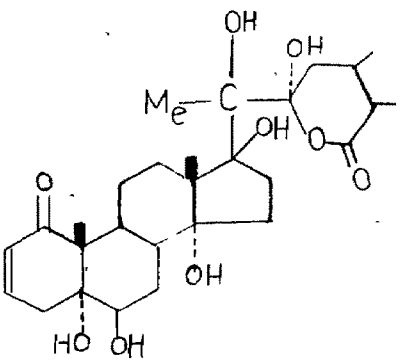
(6) Meliantriol



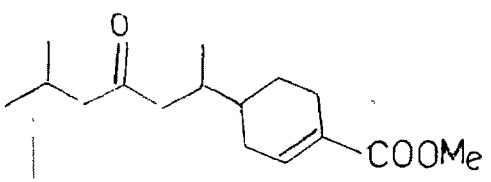
(7) Trichilin-A



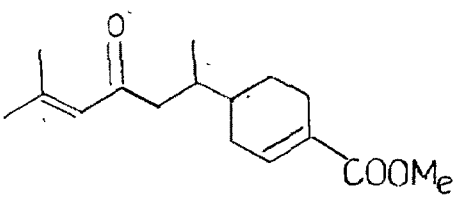
(8) Vasicinol



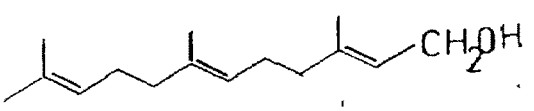
(9) Withanolide-S



(10) (+) Juvabione



(11) Dehydrojuvabione



(12) Farnesol

(family Compositae), pyrethrin-I[4](1) from this plant was the starting point for all pyrethroid insecticides and physostigmine[5](2) (from Physostigma venenosum) for the methylcarbamate insecticides. Rotenoids occuring in the genera Derris, Lonchocarpus, Tephrosia and Mundulea species (family - Leguminosae) and 'Nicotinoids' obtained from nicotiana species (family Solanaceae) are other examples of reputed plants which enjoy popularity as insecticides. The insecticidal principles of pyrethrum (pyrethrins-I & II; Cinerins-I & II and Jasmolins-I & II) rotenoids and the alkaloid "nicotine (3)" were studied exhaustively and information relating to structure activity relationships of these groups of compounds is now well documented[6,7].

However, there are some insecticidal chemicals isolated from natural sources but with a limited potential. For instance the amaroid quassin[8] (from Quassia amara) and the benzodioxole myristicin[9] is an insecticide but with a limited spectrum of activity. Diallyl disulfide[9](4), an insecticidal component of garlic also lacks the appropriate efficacy for general use as an insecticide. Unsaturated isobutyl amide such as "affinin"[10] (from Heliopsis longipes) and others are potent insect knockdown and killing agents but they are pungent and unstable compounds. Other examples of alkaloids possessing insecticidal activity are ryanodine from Ryania speciosa[11], cocculoidine and isoboldine[12] from Cocculus trilobus Solanum alkaloids, viz. tomatine, solanine and solanidine[13], stemosppironine and stemofoline from Stemona japonica[14] and pipericide from Piper nigrum[15]. Similarly a number of coumarins having different substitution patterns possess insecticidal properties[16] e.g. Pachyrrhizim (5) and erosnin from Pachyrrhizus erosus, bergapten from Orixia japonica (Rutaceae)[17].

ANTIFEEDANTS

There are number of plant extracts, volatile oils, plant materials and naturally occurring plant isolates which deter feeding of insects. A review article by Koul (1982)[18] has recorded number of such compounds which could become potential antifeedants, such as Clerodane[19-21] type from Clerodendron tricotomum, C. japonica, C. calamitosum and Caryopteris divaricata and of course the toxic terpenoids isolated from the Indian neem tree (Azadirachta indica). Azadirachtin[49], meliantriol[50] (6) and salannin[51] are the potential isolates[22-23]. Similarly three antifeedant diterpenoids have been isolated[24] from Kalamia latifolia, i.e. kalmitoxin-I, kalmitoxin-IV and grayanttoxin-III. Plumbagin[25] from Plumbago capensis and gossypol present in most of the cotton plant varieties has antifeedant properties.[26-27]

East African plants Warhurgia stuhimannii and W. ugandensis, are used in African folk medicine and food spices. Kubo and Nakanishi[28] (1977) were first to identify antifeeding activity in the extract of these plants and ultimately reported warburganal, 3-OH-warburganal, muzigadial, polygadial and ugandensidial as active ingredients. Limonoids also play role as insect antifeedant[29]. The limonoids from Trichlia roka (Meliaceae) root bark act as very effective antifeedants for Spodoptera eridania and Epilachna varivestris. The compounds like trichilin A(7),B,C,D are effective in respect of their functional groups. The furocoumarins and furoquinoline type of compounds[30] isolated from Orixa japonica (Rutaceae) act as antifeedants against S.litura. Lignanes (+) epieudesmin and (+) eudesmin from Parabenzoin praecox[31] (Lauraceae) are absolute antifeedants against S.litura at 0.05% and 1% concentrations respectively. Three alkaloids vasicine, vasicinol (8) and vasicinone

from Adhatoda vasica (Acanthaceae) have been found to possess antifeedant activity and antigonadal activity[32]. Withanolide-S (9) and withanolide-E, isolated from Physalis peruviana (Solanaceae) have been also reported[33] as good antifeedant compounds against S. littoralis larvae.

Thus number of plant extracts and phytochemicals possessing antifeedant activity provide an alternative to the use of insecticides in pest management system. Antifeedants kill insects indirectly through starvation, therefore, not harmful to parasites, predators or pollinators and also provide least chance to behave as pollutants. The use of antifeedants could also lead insects towards weeds as their food rather than economically important plants.

JUVENILE HORMONE MIMICKS

The insect also can be controlled by the use of hormone,[34,35] and the possibility of plants being one of the various sources of these third generation pesticides is rated high. Schmialek[36] made the first observation regarding juvenile hormone activity of natural product. Search for juvenile hormone analogues of plant origin received the attention after the discovery of J.H. Activity in lipid soluble material of the wood of Canadian balsam fir Abies balsamea[37,38]. The active compound (+) juvabione (10) was isolated by Bowers et al.,[39] in Maryland and Cerny et al.,[40] in Pragae and identified as the methylester of todomatuic acid. In addition to juvabione, the biologically some what more active dehydrojuvabione (11) was also isolated[40] from a Slovak fir.

High juvenile hormone activity has been also observed by Carlisle and Ellis[41] in plants like Abies nordmanniana, Pseudotsuga menziesii glauca, Tsuga canadensis. The sesquiterpenoid alcohol farnesol (12) is one of the first compound which has shown to possess the insect J.H. activity[42]. This compound occurs in nature in numerous plants. J.H. activity has been also reported in acetone extract of Iris ensata[47] and essential oil of Tagetes minuta[48] (Syn-T.glandulifera), against Dysdercus koenigii.

Bakuchiol, an isolate from the seeds of Psoralea corylfolia Linn,[43] was reported as active J.H. mimic by Joshi et al.,[44]. More potent bio-analogues were synthesised by transforming, bakuchiol into useful derivatives[45]. Koul, O. et al.,[46] has reported J.H. activity against Dysdercus koenigii in essential oil of Origanum vulgare.

From the above discussion, it is clear that there are number of plants belonging to different plant families other than listed above, which show remarkable insecticidal, antifeedant, J.H. and other pest control activities. There are many plants which are used in folk medicine and food spices. However, other reported active plants remain chemically obscure and need systematic chemical and entomological investigations. To find an economical and safe insect control agent for diverse needs of agriculture and destruction of household pests, concerted efforts are being made all over the world to discover other natural products. In this connection, it has been decided to undertake preliminary screening of extracts of various plants belonging to different families, which are known as folklore active materials. Thus, present effort has been done to evaluate such plants available in our vicinity against insects for three major activities viz. insecticidal, antifeedant and

juvenile hormones respectively and to investigate further through sequential phytochemical methods.

RESULTS AND DISCUSSIONS

In all 56 plants* belonging to 33 plant families were biologically evaluated for the insecticidal, juvenile hormone mimicking and antifeedant activities. All the plant materials were extracted in a soxhlet at 80-90°C, successively with three different solvents with increasing polarity, i.e. petroleum ether (60-80), dichloroethane and ethanol, to get hydrocarbons, non-polar compounds and fatty materials in petroleum ether, more polar compounds in dichloroethane and most polar compounds in ethanol extract. All the three plant extracts of all the plants were subjected to three types of evaluations. Insecticidal activity was evaluated against housefly (Musca domestica), juvenile hormone activity against red cotton bugs (Dysdercus koenigii) and antifeedant activity against tobacco armyworms (Spodoptera litura). For initial evaluation all the three extracts of each plant were taken for testing at a standard concentration of 5% (w/v) in solvent. In case of insecticidal activity more than 40% mortality was denoted as positive effect. In case of juvenile hormone activity the percentage inhibition value was based on the formation of 6th instar only. More than 30% of 6th instar formation was considered as positive inhibition. While in case of antifeedant activity choice and no-choice tests were performed and in both the experiments, percentage deterrence of more than 50% was recorded as positive effect. Data of percentage yield of petroleum ether, dichloroethane and ethanol extracts of plants and its entomological test report is given in Table-1. List of the plants possessing different activities is given below.

Insecticidal activity

(1) Gloriosa superba Linn. (2) Rhododendron arboreum Sm.

(3) Thevetia neriifolia Juss. (4) Ziziphus xylopyrus Willd.

* All the plants were identified by our botanist.

TABLE - 1 : EXTRACTION OF PLANTS AND BIOLOGICAL TESTING OF PLANT EXTRACTS.

Sr. No.	Name of the plant used (Family)	Part of the plant used for the extraction (Wt. of the plant material taken) (g)	Solvent/ Wt. of the extract (g) (% Yield)	ACTIVITY Insecticidal	J.H.	Anti-feedant	Remarks
01.	Aerua javanica Juss. (AMARANTHACEAE)	Herbage (429)	1/8.3 (1.93)	-	+	+	
			2/2.3 (0.53)	-	+	+	
			3/14.6 (3.4)	-	-	-	
02.	Aerua javanica Juss. (AMARANTHACEAE)	Whole plant (241)	1/6.3 (2.61)	-	+	+	
			2/1.5 (0.62)	-	+	+	
			3/18 (7.46)	-	-	+	
03.	Adhatoda vasica Nees. (ACANTHACEAE)	Herbage (250)	1/ 3 (1.2)	-	-	+	
			2/ 1.1 (0.44)	-	-	+	
			3/ 8.0 (3.2)	-	-	+	
04.	Alangium lamarckii Thw. (ALANGIACEAE)	barks (170)	1/ 0.26 (0.15)	-	-	-	
			2/ 0.56 (0.33)	-	-	+	
			3/ 0.914 (0.53)	-	-	-	
05.	Alangium lamarckii Thw. (ALANGIACEAE)	Fruits (158)	1/ 1.2 (0.76)	-	-	-	
			2/ 0.81 (0.51)	-	-	-	
			3/40.0 (25.31)	-	-	-	
06.	Ammannia baccifera Linn (LYTHRACEAE)	Whole Plant (400)	1/ 7 1 (1.77)	-	-	-	
			2/ 4.02 (1.0)	-	-	-	
			3/38.8 (9.7)	-	-	-	
07.	Asphodelus tenuifolius Cav. (LILIACEAE)	Whole plant (15)	1/ 0.3 (2.0)	-	-	-	
			2/ 0.12 (0.8)	-	-	-	
			3/ 1.2 (8.0)	-	-	+	
08.	Bignonia illicium (BIGNONIACEAE)	Stems & leaves (285)	1/ 5.1 (1.78)	-	-	-	
			2/ 2.7 (0.94)	-	-	-	
			3/11.0 (3.85)	-	-	-	
09.	Blumea alata DC. (COMPOSITAE)	Herbage (150)	1/ 4.4 (2 9)	-	-	+	
			2/ 2.4 (1.6)	-	-	-	
			3/ 9.02 (6.0)	-	-	-	
10.	Blumea lecera DC. (COMPOSITAE)	Herbage (260)	1/5.38 (2.06)	-	-	-	
			2/2.6 (1.0)	-	-	-	
			3/11 0 (4.23)	-	-	-	

Sr No	Name of the plant used (Family)	Part of the plant used for the extraction (Wt. of the plant material taken) (g)	Solvent/ Wt of the extract (g) (% Yield)	ACTIVITY Insecticidal	J H.	Anti-feedant	Remarks
11.	Boenninghausenia albiflora Rchb (RUTACEAE)	Whole Plant (117)	1/1.5 (1.28)	-	-	-	
			2/ 0.5 (0 42)	-	-	-	
			3/ 7.0 (5.9)	-	-	-	
12.	Cassia fistula Linn. (LEGUMINOSAE)	Fruits (300)	1/ 0.22 (0.07)	-	-	-	
			2/ 1.73 (0.57)	-	-	-	
			3/35.0 (11.66)	-	-	-	
13.	Chrozophora plicata A.Juss. (EUPHORBIACEAE)	Whole Plant (213)	1/14.0 (6.5)	-	-	-	
			2/ 1.0 (0.46)	-	-	-	
			3/16 0 (7 51)	-	-	-	
14.	Cinnamomum tamala Fr. Nees. (LAURACEAE)	Leaves (50)	1/ 2.0 (4.0)	-	-	+	
			2/ 0.5 (1.0)	-	-	-	
			3/ 5.0 (10 0)	-	-	-	
15.	Codiaeum variegatum Bl. (EUPHORBIACEAE)	Leaves (140)	1/ 4.0 (2.85)	-	-	+	
			2/ 1.1 (0.78)	-	-	-	
			3/16.0 (11.42)	-	-	-	
16.	Croton sparsiflorus Linn. (EUPHORBIACEAE)	Herbage (250)	1/ 6.7 (2.68)	-	-	-	
			2/ 2.5 (1.0)	-	-	-	
			3/19.0 (7.6)	-	-	-	
17.	Delphinium elatum Linn. (RANUNCULACEAE)	Whole Plant (60)	1/ 0.8 (1.3)	-	-	+	Growth inhibition observed.
			2/ 0.85 (1.36)	-	-	-	
			3/ 6.5 (10.83)	-	-	-	
18.	Duranta plumieri Jacq. (VERBENACEAE)	Whole Plant (265)	1/ 0.5 (1.88)	-	-	-	
			2/ 0.5 (1.88)	-	-	-	
			3/ 5.0 (18.83)	-	-	-	
19.	Duranta plumieri Jacq. (VERBENACEAE)	Seeds (405)	1/ 3.5 (0.86)	-	-	-	
			2/ 2.5 (0.61)	-	-	-	
			3/20.0 (4.9)	-	-	-	
20.	Dysoxylum malabaricum Bedd. (MELIACEAE) (Oleoresin)	Woods	Acetone	-	-	+	

Sr. No.	Name of the plant used (Family)	Part of the plant used for the extraction (Wt. of the plant material taken) (g)	Solvent/ Wt. of the extract (g) (% Yield)	A C T I V I T Y Insecticidal	J H.	Anti-feedant	Remarks
21.	Ganoderma lucidum Fungi (BASIDOMYLTAE)	Fruiting bodies (668)	1/ 2.5 (0.37)	-	-	-	
			2/ 3.43 (0 513)	-	-	-	
			3/ 5 62 (0 84)	-	-	-	
22.	Gardenia lucida Roxb. (RUBIACEAE)	Resin (25)	Acetone/ 2.5 (10.0)	-	-	-	
23.	Gloriosa superba Linn. (LILIACEAE)	Roots (160)	1/ 1.0 (0.62)	-	-	-	Antigonadal activity was observed in PE. and DCE. extracts.
			2/ 0 6 (0.37)	+	-	+	
			3/ 6.1 (3.8)	+	-	+	
24	Gynandropsis pentaphylla DC (CAPPARIDACEAE)	Whole Plant (185)	1/ 1.2 (0.64)	-	-	-	
			2/ 2.2 (1.18)	-	-	+	
			3/26.0 (14.0)	-	-	-	
25.	Hedychium spicatum Ham. (ZINGIBERACEAE)	Roots (141)	Acetone/ 4.4 (3.12)	-	-	-	
26.	Hyptis suaveolens poit. (LABIATAE)	Herbage (115)	1/ 3.1 (2 69)	-	-	-	
			2/12.0 (10.4)	-	-	-	
			3/ 7.0 (6.08)	-	-	-	
27.	Jatropha curcas Linn. (EUPHORBIACEAE)	Seeds (160)	1/50.0 (31.2)	-	-	-	
			2/ 0.9 (0.56)	-	-	-	
			3/ 4.9 (3.06)	-	-	-	
28.	Jatropha gossypifolia Linn. (EUPHORBIACEAE)	Whole Plant (160)	1/ 1.0 (0.62)	-	-	-	
			2/ 0.6 (0.37)	-	-	-	
			3/ 6.1 (3.8)	-	-	+	
29.	Launaea nudicaulis Hk.f. (COMPOSITAE)	Herbage (160)	1/ 5.2 (3.25)	-	-	-	
			2/ 2.6 (1.6)	-	-	-	
			3/ 8.82 (5.51)	-	-	-	
30.	Lagenaria leucantha Rusby. (CUCURBITACEAE)	Fruits (160)	1/ 5 2 (3.25)	-	-	-	
			2/ 2.6 (1.6)	-	-	-	
			3/ 8.8 (5.5)	-	-	-	
31.	Leucas aspera Spreng. (LABIATAE)	Whole Plant (90)	1/ 0.23 (0.25)	-	-	-	
			2/ 0.164 (0.18)	-	-	-	
			3/ 0.21 (0.237)	-	-	-	

Sr. No.	Name of the plant used (Family)	Part of the plant used for the extraction (Wt. of the plant material taken) (g)	Solvent/ Wt. of the extract (g) (% Yield)	A C T I V I T Y Insecticidal	J.H.	Anti-feedant	Remarks
32.	Luvunga scandens Ham. (RUTACEAE)	Fruits (540)	1/122 (22.59)	-	-	-	
			2/ 9.0 (1.66)	-	-	-	
			3/50.0 (9.2)	-	-	-	
33.	Melia azedarach Linn. (MELIACEAE)	Flowers (137)	1/ 2 1 (1.53)	-	-	+	Growth inhibitory activity was observed in DCE extract.
			2/ 1.4 (1.02)	-	-	+	
			3/18.0 (13.13)	-	-	+	
34.	Melia azedarach Linn (MELIACEAE)	Fruits (226)	1/ 7.5 (3 318)	-	-	+	
			2/ 1.16 (0.51)	-	-	+	
			3/28.0 (12.38)	-	-	+	
35.	Millettia ovalifolia Kurz (LEGUMINOSAE)	Flowers (204)	1/ 2.8 (1.31)	-	-	-	
			2/ 0.75 (0.367)	-	-	-	
			3/19.0 (9.3)	—	—	—	
36.	Millettia ovalifolia Kurz. (LEGUMINOSAE)	Seeds (275)	1/48.0 (17.45)	-	-	-	
			2/ 1.4 (0.5)	-	-	-	
			3/ 9.0 (3.2)	-	-	-	
37	Mimusops hexandra Roxb (SAPOTACEAE)	Bark of stems (275)	1/ 5.0 (1.8)	-	-	-	
			2/ 0.8 (0.29)	-	-	-	
			3/35.0 (12.7)	-	-	-	
38.	Moringa oleifera Lam. (MORINGACEAE)	Fruits (250)	1/ 0.21 (0.084)	-	-	-	
			2/ 0.51 (0.2)	-	-	-	
			3/ 0.57 (0.228)	-	-	+	
39.	Nerium odorum Soland (APOCYNACEAE)	Herbage (380)	1/16.2 (4.26)	-	-	-	
			2/16.05 (4.22)	-	-	-	
			3/46.5 (12.23)	-	-	+	
40.	Nyctanthes arbortristis Linn. (OLEACEAE)	Whole Plant (323)	1/ 7.1 (2.19)	-	-	-	
			2/ 2.3 (0.71)	-	-	-	
			3/31.0 (9.59)	-	-	-	
41	Oligochaeta ramosa wagon (COMPOSITAE)	Herbage (430)	1/ 2.7 (0.62)	-	-	-	
			2/0.8 (0.186)	-	-	-	
			3/12.0 (2 79)	-	-	-	
42.	Orobanche aegyptiaca Pers. (OROBANCHACEAE)	Whole Plant (310)	1/ 2.0 (0 64)	-	-	-	
			2/ 0 3 (0.096)	-	-	-	
			3/ 4.0 (1.29)	-	-	-	

Sr. No.	Name of the plant used (Family)	Part of the plant used for the extraction (Wt. of the plant material taken) (g)	Solvent/ Wt. of the extract (g) (% Yield)	A C T I V I T Y Insecticidal	J H	Anti-feedant	Remarks
43.	Papaver somniferum Linn. (PAPAVERACEAE)	Opium Mark (64)	1/16.0 (25.0)	-	-	-	
			2/ 1.5 (2.3)	-	-	-	
			3/ 2.1 (3 2)	-	-	-	
44.	Paspalum scrobiculatum Linn. (GRAMINEAE)	Grain husk (380)	1/ 1.12 (0.29)	-	-	-	
			2/0.7 (0.18)	-	-	-	
			3/ 1.5 (0.39)	-	-	-	
45.	Pavonia odorata Willd. (MALVACEAE)	Roots (346)	1/ 6.1 (1.763)	-	-	-	Insect growth regulating activity was observed in PE extract.
			2/ 5.22 (1.5)	-	-	-	
			3/40.0 (11.56)	-	-	-	
46.	Plumbago zeylanica Linn. (PLUMBAGINACEAE)	Herbage (241)	1/ 6.3 (2.6)	-	-	+	
			2/ 1.5 (0.62)	-	-	+	
			3/18.6 (7.71)	-	-	-	
47.	Plumbago zeylanica Linn. (PLUMBAGINACEAE)	Inflore-scence (300)	1/10.2 (3.4)	-	-	-	
			2/ 3.1 (1.03)	-	-	-	
			3/ 8.0 (2 66)	-	-	-	
48.	Plumbago zeylanica Linn. (PLUMBAGINACEAE)	Roots (250)	1/ 0.45 (0.18)	-	-	+	
			2/ 0.3 (0.12)	-	-	-	
			3/15.0 (6.0)	-	-	-	
49.	Ramalina subcomplanta (LICHEN)	Whole Plant (52)	1/ 0.66 (1.26)	-	-	+	Growth is inhibited.
			2/ 0.76 (1 46)	-	-	-	
			3/ 2.91 (5.59)	-	-	+	
50.	Randia dumetorum Poir. (RUBIACEAE)	Fruits (209)	1/ 1 9 (0.9)	—	+	-	Activity not consistent.
			2/ 0.9 (0.43)	-	-	-	
			3/50.0 (23.92)	—	-	-	
51	Rhododendron arboreum Sm. (ERICACEAE)	Herbage (100)	1/ 6.0 (6.0)	+	-	-	
			2/ 2.0 (2.0)	+	-	-	
			3/10.0 (10.0)	-	-	-	
52.	Rubia cordifolia Linn. (RUBIACEAE)	Stems (187)	1/ 2.0 (1.06)	-	-	-	Some repellent activity was observed against flies.
			2/ 1.0 (0.53)	-	-	-	
			3/ 3.7 (1.97)	-	-	-	

Sr. No.	Name of the plant used (Family)	Part of the plant used for the extraction (Wt of the plant material taken) (g)	Solvent/ Wt. of the extract (g) (% Yield)	ACTIVITY Insecticidal	J.H.	Anti-feedant	Remarks
53	Saccharum spontaneum Linn (GRAMINEAE)	Leaves (200)	1/3.1 (1.55)	-	-	-	
			2/ 1.0 (0.5)	-	-	-	
			3/10.5 (5.25)	-	-	-	
54.	Schima wallichii Korth. (THEACEAE) Nepal	Leaves + stems (292)	1/15.09 (5.16)	-	-	-	Growth inhibition observed
			2/ 3.1 (1 06)	-	-	-	
			3/20.0 (6.84)	-	+	-	
55.	Streblus asper Lour. (MORACEAE)	Whole Plant (380)	1/ 7.7 (2.02)	-	-	-	
			2/ 1.75 (0.46)	-	-	-	
			3/14.0 (3 68)	-	-	-	
56.	Streblus asper Lour. (MORACEAE)	Herbage (400)	1/ 8 0 (2.0)	-	-	-	
			2/ 2.0 (0.5)	-	-	-	
			3/16.0 (4.0)	-	-	-	
57.	Terminalia belerica Roxb. (COMBRETACEAE)	Seed kernals (190)	1/ 0.45 (0.236)	-	-	-	
			2/ 0.3 (0.15)	-	-	+	
			3/52.0 (27.36)	-	-	-	
58.	Thevetia nerifolia Juss. (APOCYNACEAE)	Herbage (220)	1/10 1 (4.59)	+	-	-	
			2/ 0.7 (0.318)	-	-	-	
			3/17.0 (7.72)	-	-	-	
59.	Usnea lucea (LICHEN)	Whole Plant (489)	1/ 5.4 (1.1)	-	-	+	
			2/ 4.0 (0.81)	-	-	-	
			3/25.0 (5.11)	-	-	-	
60.	Vitex nugundo Linn. (VERBENACEAE)	Whole plant (190.4)	1/ 3.3 (1.73)	-	-	-	
			2/ 0.3 (0.15)	-	-	-	
			3/12.0 (6.28)	-	-	-	
61.	Vittadinia australis A. Rich (COMPOSITAE)	Herbage (200)	1/ 2.4 (1.2)	-	-	+	
			2/ 1.2 (0.6)	-	-	+	
			3/ 5.1 (2.55)	-	-	-	
62.	Xanthum strumarium Linn. (COMPOSITAE)	Roots (445)	1/ 0.15 (0.0339)	-	-	-	
			2/ 0.82 (0.184)	-	-	-	
			3/ 8.2 (1.84)	-	-	-	

Sr. No.	Name of the plant used (Family)	Part of the plant used for the extraction (Wt. of the plant material taken) (g)	Solvent/ Wt. of the extract (g) (% Yield)	ACTIVITY Insecticidal	J.H.	Anti-feedant	Remarks
63.	Zanthoxylum armatum DC. (RUTACEAE)	Fruits (140)	1/12.0 (8.57)	-	+	-	
			2/ 1.5 (1.07)	-	-	-	
			3/ 9.0 (6.42)	-	-	-	
64.	Zanthoxylum rhetsa DC (RHAMNACEAE)	Fruits (200)	1/ 5.5 (2.7)	-	-	-	Growth inhibition observed.
			2/ 2.2 (1.14)	-	-	-	
			3/ 6.0 (3.0)	-	-	-	
65.	Ziziphus xylopyrus Willd. (RHAMNACEAE)	Fruits (150)	1/ 2.0 (1.33)	+	+	-	
			2/ 0.85 (0.56)	-	-	-	
			3/61.0 (40.6)	-	-	-	

ABBREVIATIONS + Active, - Non active

(1) Pet.ether extract (PE.) (2) Dichloroethane extract (D.C.E.)

(3) Ethanol extract (4) Juvenile hormone (JH)

Note : All the plant materials were extracted for 10-16 hrs with above mentioned solvents.

Juvenile Hormone activity

(1) Aerua javanica Juss.
(2) Randia dumetorum Poir.
(3) Schima wallichii Korth
(4) Zanthoxylum armatum DC.
(5) Ziziphus xylopyrus Willd.

Antifeedant activity

(1) Aerua javanica Juss.
(2) Adhatoda vasica Nees.
(3) Alangium lamarckii Thw.
(4) Asphodelus tenuifolius Cav.
(5) Blumea alata DC.
(6) Cinnamomum tamala Fr. Nees.
(7) Codiaeum variegatum Bl.
(8) Delphinium elatum Linn.
(9) Dysoxylum malabaricum Bedd.
(10) Gloriosa superba Linn.
(11) Gynandropsis pentaphylla DC.
(12) Jatropha gossypifolia Linn.
(13) Melia azedarach Linn.
(14) Moringa oleifera Linn.
(15) Nerium odorum Soland.
(16) Plumbago Zeylanica Linn.
(17) Ramlina subcomplanta
(18) Terminalia belerica Roxb.
(19) Usnea lucea
(20) Vittadinia australis A. Rich.

Five different samples of Randia dumetorum fruits were collected from local market and continuously extracted in a soxhlet for 12 hrs with petroleum ether (60-80), which after solvent removal gave yellow viscous oil (Table-2). Petroleum ether extract of all the five samples were tested against Dysdercus koenigii (red cotton bugs) for juvenile hormone activity.

Table-2 Extraction of Randia dumetorum (fruits)

Sr. No,	Wt. of the fruit.	Solvent/Time (hr)	Wt. of the extract (g)	% yield	Remarks
01.	209	Petroleum/12 ether	1.9	0.9	J.H. active
02.	323	..do..	1.41	0.43	Not active
03.	570	..do..	4.00	0.7	J.H. active
04	618	..do..	6.1	1.00	..do..
05.	105	..do..	0.86	0.82	Not active.

The activity was observed in three out of five samples tested and reason for nonreproducibility of juvenile hormone activity in different samples remains a question to be solved. The active extract was chromatographed over SiO_2 gel column and eluted with petroleum ether-acetone mixture of various percentage. Total 13 fractions were collected (monitored by TLC) and tested for juvenile hormone activity, and only one fraction (69 mg from 5.5 gm extract) was found to be active. Active fraction was very complex mixture of many compounds (TLC), and there was no significant enhancement in activity as compared to the total extract of the plant was observed, so further work was discontinued.

The dried roots (8.1 kg) of Analangium lamarckii (collected from our campus) were extracted with hot acetone in a big soxhlet. The acetone extract was adsorbed over mixture of Na_2SO_4 plus celite and further extracted successively with hot petroleum ether and dichloroethane in a soxhlet. Sovent removal furnished 100 g (1.2%) petroleum ether extract and 19.2 gm (0.23%) dichloroethane extract. Only dichloroethane extract was active against Spodoptera litura (tobacco armyworms). Therefore, dichloroethane extract (3.7 g) was subjected to broad cut chromatography over SiO_2 gel and eluted with mixture of ethylacetate and ethanol. Total four fractions were collected. Out of four fractions, three fractions were found to be active. The major and active fraction-1 (1.5 gm) was taken into solvent ether and insoluble portion was filtered through filter paper. Ether soluble portion after solvent removal was triturated with petroleum ether and separated into ether soluble and petroleum ether soluble portion. Thus three materials, i.e. petroleum ether soluble (823 mg), solvent ether soluble (478 mg) and solvent ether insoluble (117 mg) were made. Amongst these only ether soluble portion was found

to be active. The ether soluble portion was further subjected to column chromatography over SiO_2 gel and eluted with the mixture of pet.ether acetone. Major seven fractions were collected and all were tested for antifeedant activity. Three fractions out of seven fractions exhibited antifeedant activity, but all the three fractions were mixtures. There was no significant enhancement in activity in any of the fractions compared to the total extract was observed, so further work was discontinued.

EXPERIMENTAL

For general remarks refer experimental of Chapter-1.

Genral procedure for extraction of various plants.

Different plant materials collected from various places were washed, cut and dried in a shade at room temperature for 15-20 days depending on the plant material and powdered finely. Each plant material was extracted successively in a soxhlet with petroleum ether, dichloroethane and ethanol on a waterbath (90-95°C) for 10-16 hrs. depending on the plant material. All the three extracts were collected separately after the solvent removal under reduced pressure (60°/100 mm) on rotary evaporator and dried. The details of its results are reported in Table-1.

Large scale extraction of Alangium lamarckii roots

The roots of Alangium lamarckii were collected from our campus, washed, cut and dried at room temperature for 15 days. The dried roots were powdered and 8.1 kg of root powder was extracted with technical grade hot acetone (160 L) in a big soxhlet continuously for 4 days. The solvent was removed from the extract to get 225 gm dark brown oily material. The crude extract was adsorbed over celite (500 gm) and the powder made thicker with sodium sulphate (1 kg). This was successively extracted with hot petroleum ether followed by dichloroethane for 24 hrs. each. The solvent was removed separately from both the extracts to give 100 gm (1.2%) dark brown viscous oil from petroleum ether extract and 19.2 gm (0.237%) dark brown gummy solid from dichloroethane extract. Only dichloroethane extract exhibited antifeedant activity. It was subjected to broad cut chromatography over silica gel.

Table-3 Chromatogram

Material 3.7 gm adsorbed over 7 gm silica gel.

Adsorbent 600 gm SiO_2 (II b)

Column dimension 6 cm x 50 cm

Fr. No.	Eluent	Vol. of fraction	Wt. of fractoin (g)	Remarks
01.	2 % Ethanol in EtOAc	500 ml x 6	1.5031	Active fraction-1
02.	10% Ethanol in EtOAc	..do..	0.7012	..do..
03.	25% Ethanol in EtOAc	..do..	0.6831	Inactive fraction
04.	35% Ethanol in EtOAc	..do..	0.7135	Active fraction
		Total :	3.6009	

All the fractions were monitored by TLC (solvent system, 75% EtOAc in ethanol).

Further separation of active fraction-1

Active fraction-1 (1.5 gm) was taken in solvent ether (15 ml), insoluble portion was filtered through filter paper. The insoluble portion from the filter paper was recovered with methanol (10 ml) and distillation of solvent gave 117 mg solid. Ether soluble portion after distillation of solvent gave viscous liquid. This was triturated with petroleum ether (10 ml x 3) and distillation of solvent gave 823 mg gummy liquid. Thus, three fractions i.e. petroleum ether soluble (823 mg), ether soluble (478 mg) and solvent ether insoluble (117 mg) were separated. Only ether soluble portion exhibited antifeedant activity and it was further chromatographed over silica gel.

Table-4 Chromatogram

Material 478 mg, adsorbed over 1 gm silica gel.

Adsorbent 13 gm silica gel, grade II b.

Column dimension 40 cm x 1 cm.

All the fractions were monitored by TLC (solvent system pet. ether-acetone (1:1).

Fr. No.	Eluent	Vol. of fraction	Wt. of fraction (gms)	Remarks
1-3	20 % Acetone in Pet.Ether	100mlx3	0.1081	
4-5	30 % Acetone in Pet.Ether	100mlx2	0.0512	Active fraction.
6	40 % Acetone in Pet.Ether	100mlx1	0.0613	
7-8	50 % Acetone in Pet.Ether	100mlx2	0.0458	Active fraction.
9-14	60 % Acetone in Pet.Ether	100mlx6	0.0452	
15-18	75 % Acetone in Pet.Ether	100mlx4	0.0421	Active fraction.
19-20	Ethanol	100mlx2	0.1103	
	T O T A L		0.4640	

Column chromatography of petroleum ether extract of Randia dumetorum fruits

Table-5 Chromatogram

Material 5.5 gm Adsorbed over 8 gm silica gel.

Adsorbent 320 gm silica gel (II b)

Column dimension 58 cm x 3.6 cm

Fractions were monitored by TLC (solvent system, 6% acetone in Pet.Ether)

Fr. No.	Eluent	Vol. of fraction	Wt. of fraction (gms)	Remarks
1-3	Pet.Ether	250mlx3	0.0534	
4-5	Pet.Ether	250mlx2	0.0073	
6-11	1% Acetone in Pet.Ether	250mlx6	0.4765	
12-13	1% Acetone in Pet.Ether	250mlx2	1.1423	
14-16	2% Acetone in Pet.Ether	250mlx3	0.0926	
17-18	2% Acetone in Pet.Ether	250mlx2	0.0859	
19-22	3% Acetone in Pet.Ether	250mlx4	0.0639	J.H. active fraction
23-26	3% Acetone in Pet.Ether	250mlx4	0.2977	
27-29	3% Acetone in Pet.Ether	250mlx3	0.0719	
30-34	5% Acetone in Pet.Ether	250mlx5	0.2271	
35-37	10% Acetone in Pet.Ether	250mlx3		
38-43	10% Acetone in Pet.Ether	250mlx6	0.4650	

Fr. No.	Eluent	Vol. of fraction	Wt. of fraction (gms)	Remarks
44-46	20% EtOAc in Pet.Ether	250mlx3	0.3142	
47-52	Ethanol	250mlx6	2.0006	
	T O T A L :		5.2984 gm	

INSECT BIOASSAYS

All the three plant extracts were subjected to three types of evaluations, namely insecticidal, juvenile hormone mimicking and antifeedant activities. For initial evaluation all the extracts were taken for testing at a standard concentration of 5% w/v in solvent.

Insecticidal activity

Test insect for this activity was housefly (Musca domestica). The flies were taken from our routine cultures maintained in the laboratory at 27±1°C, 70±5% RH and 16h photophase. Only female houseflies were used and applied topically with 1 µL dose of test material with the help of an Arnold's microapplicator. The controls received 1 ml of solvent alone. In each test 3 replicates of 10 flies each were taken and provided with normal food after treatment. After 24 hours the mortality was recorded to calculate percentage against controls. If mortality was more than 40%, it was denoted as positive effect, otherwise, the test materials were considered to be inactive.

Juvenile hormone activity

This type activity was evaluated against red cotton bugs (Dysdercus koenigii). The 5th instar freshly moulted larvae were taken from our routine

cultures as mentioned above. The larvae were starved for about 20 hours (provided with only drinking water) followed by the topical application of the test materials (2 Ml/larva) with the help of microapplicator. After treatment the larvae were allowed to grow on normal food (cotton seeds) till next moulting. After next moulting the number of 6th instar adultoids and adults were counted to calculate percentage inhibition due to treatment. In each test 3 replicates of 10 insects were used and controls were applied with solvent alone. The percentage inhibition value was based on the formation of 6th instars only. More than 30% of 6th instar formation was considered as positive inhibition. Antifeedant activity - refer experimental of Chapter-2.

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ABSTRACT

Insecticidal, Antifeedant and Juvenile hormone activity was tested in 189 extracts of 56 plants belonging to 33 different families. It was tested against Musca domestica (housefly), Spodoptera litura (tobacco armyworms) and Dysdercus koenigii (red cotton bugs) respectively. Insecticidal activity was observed in 4 plants, while antifeedant and juvenile hormone activity was observed in 20 and 5 plants respectively. Detailed investigation of juvenile hormone active plant - Randia dumetorum and antifeedant active plant - Alangium lamarckii has been described.