Himalayan Plant Species as Pesticidal Agents

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Abstract: The control of diseases and insect, pests for securing world is important issue for us. A significant way to find a better treatment for crop is the search for insecticidal, pesticidal and antimicrobial constituents. From many years research in biology and biochemistry has made it possible a complete new approach for the protection of plants. As part of our Botanical pesticide research project, numerous compounds from Himalayan plant species of Uttarakhand, India with potential antifeedant activity have been identified. The plants selected for complete investigations were *Boenninghausenia albiflora*, *Skimmia anquetillia*, *Glycosmis arborea*, *Vitex negundo*, *Premna barbata*, *Callicarpa arborea* and *Clerodendron indicum* and the pests selected were the *Plecoptera reflexa*, the popular defoliator *Clostera cupreta*, bamboo leaf roller *Crypsiptya coclesalis* and polyphagous pest *Spodoptera litura*. The extracts and pure compounds were also tested against plant pathogens, *Agrobacterium tumifaciens*, *Pseudomonas syringae* and *Pactobacterium carotovorum* for antibacterial activity. In all tested plants *B. albiflora* and *S. anquetillia* showed potent insecticidal and antibacterial properties. In addition all investigated plants of Rutaceae and Verbenaceae family were found to be active against *S. litura* pest.

Keywords: Botanical pesticides, Boenninghausenia albiflora, Callicarpa arborea, Clerodendron indicum, Glycosmis arborea, Premna barbata, *Skimmia anquetillia*, Vitex negundo.

1. INTRODUCTION

Botanical pesticides are the compounds that are derived from plants and have been in use from a long period for pest control. Pyrethrum, rotenone, nicotine, sabadilla, and quassin are the only botanical pesticides used in the Western Hemisphere until the Second World War. However, after the Second World War (1939 to 1945), they lost their importance with the discovery of synthetic chemicals insecticides in the mid 1930s to 1950s [1]. Synthetic pesticides have rapid action on pests but they are often toxic to human and animals [2, 3], nondegradable, environmental persistent and also effects on non targeted organisms [4]. Some synthetic pesticides like methyl bromide participates in ozone layer depletion [5], so more target selective and biodegradable pesticides are required to replace the environmentally persistent chemicals with broad spectrum toxicity [6]. Botanical insecticides are often effective alternative to organic pesticides. In 1950, Heal *et al*. [7] reported approximately 2500 plants in 247 families with some sort of toxic properties against insects. Simmond *et al*. [8] identified 59 plant families having potent insecticidal activity and more than 2400 plants species containing pesticidal properties. Among the all studied plant families Meliaceae, Rutaceae, Asteraceae, Labiateae, Piperaceae and Annonaceae having promising activity against pests and plant pathogens [9].

There are different categories of plant secondary metabolites including alkaloids, terpenoids, steroids, phenolics and cardiac glycosides, aryl polysulphides, benzonoids, quinoids, flavonoids, coumarins, sugars etc. These substances enhance the activity of plant against insects and also reduce the rate of resistance development by the pests against pesticides [10, 11]. Alkaloids, quinones, terpenes and flavones are the major chemical groups seem to be leading groups in biological activities [12].

Flavones belong to the polyphenolic groups and are essential for plant growth, development and defense against infection or injuries by pests. About 3000 verities of flavones are known to date [13], with several biological properties. Flavones from aerial parts of *Flourensia oolepis* [14], *Ajuga nipponensis* [15], are cause of strong antifeedant activity against the pests and striped flea beetles respectively. Coumarin is a type of flavone that is less studied, but also possesses antifeedant and insecticidal activity [16]. The structures of active and inactive coumarins as isoimperatorin and imperatorin or xanthotoxin are compared, substitution of groups at different position (C-4 or C-9) account from the differences in antifeedant activity (Fig. **1**). Some evidences suggested that flavones may the slow capability of insects to habituate [17]. Almost all flavones are insect feeding deterrent.

Terpene is a largest class of natural products and was demonstrated to be toxins, repellents or attractants to other organisms [18-20]. Clerodane diterpenoids are well known insect antifeedant [21]. Jodrelin and scutalpin isolated from *Scutellaria* are much active neo-clerodanes against pests [22]. Monoterpenes isolated from ***peppermint*** (*Mentha piperita*) had been reported for the insecticidal activity [23]. Terpene as pyrethroid disrupts the insect nervous system by acting on the voltage-sensitive sodium channel protein of the nerve membrane [24]. By inducing repetitive discharge in nerves in place of single impulses, the nervous system becomes hyperexcited, which results in rapid, uncoordinated movement and paralysis in target organisms. Most of triterpenes having antifeedant activity against insects and pests they reduces feeding by acting on central nervous system.

Alkaloid is heterogeneous group of alkaline, organic, compounds containing nitrogen and usually oxygen, possess various biological activities. They are produced by a large variety of organisms, including bacteria, fungi, plants, and animals. Alkaloids in plants have been also reported as antifeedant and insecticidal agents, sesquiterpene pyridine alkaloids from *Maytenus chiapensis* reported to strong antifeedant activity against *Spodoptera littoralis* [25], other alkaloids from *Erythrina latissima* [26], *Stemona japonica* [27] and *Fagara macrophylla* [28] against *Spodoptera littoralis*, *Spodoptera frugiperda* and other relevant agricultural insect, pests. Antimicrobial alkaloids were also reported from *Zanthoxylum teteraspermum* and *Zanthoxylum caudatum* [29]. Veratrum alkaloids as veratridine, cevadine and sabadilla are usually applied as an extract (sabadilla) against insects. The mode of action of the veratrum alkaloids is similar to that of the pyrethroids.

A quinone is a class of organic compounds that are formally derived from aromatic compounds. Quinones are well known for their antimicrobial properties, juglone and lapachol from walnut and alkanet reported for antibacterial and antifungal activities [30-32]. Nortriterpene quinone from some *Maytenus* Species (Celastraceae) was reported to active against Codling Moth, *Cydia pomonella* [33]. Prenylated quinones of *Baja California* and *Baja Chihuahua* are reviewed to defense against phytophagous insects and pathogens [34]. Quinones act as respiration inhibitors on fungi. Naphtaquinones are act as growth inhibitors, some quinones altered metabo-

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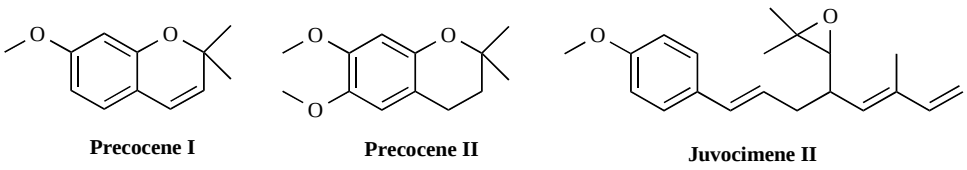

Fig. (1). Structures of anti-juvenile hormonal compounds from plants.

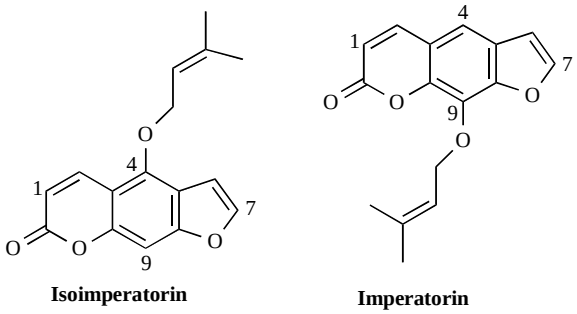

Fig. (2). Structures of active and inactive coumarins.

lism leading to disturbance in the feedback mechanism exist between insect neuroendocrine system, fat body and gonads [35].

Juvenile hormone plays a crucial role in the regulation of the molting and metamorphosis processes in insects. A special category of compounds that disrupts insect juvenile and molting hormone activity had been previously discovered from plants [36] these type of anti-juvenile hormonal compounds are called juvenoids. The treatment of crops with juvenile hormone showed inhibition in normal growth of insects and results in their death. Juvocimene II form *Ocimum basilicum* [37] and prococenes I and II from *Ageratum houstonianum* (Asteraceae) (Fig. **2**) act as antijuvenile hormonal compounds that include a lethal, precocious metamorphosis in immature stages of many insects and also makes sterilize the adult females [38].

2. ROLE OF BOTANICAL PESTICIDES IN AGRICULTURE

About 30% of world crop production loss is mentioned by pests, insects and microorganisms [39]. Agricultural tropical countries as India suffer from severe loss of agriculture crops by pests. In India every year Rs. 25, 000 - 50, 000 crore worth of crops is destroyed by pests and insects. Farmers spend excess of their income in synthetic pesticides to get rid of these natural enemies. Synthetic pesticides not only effects on pests but also produce harmful effects on environment and human health. Synthetic pesticides affected more than thirty lac people all over the world from which more than 2.2 lac people have died due to illness as cancer, hepatic disorders and nervous disability produced by synthetic pesticides, these are also cause of infertility and blindness in mammals. Insect and pests also developed resistance against these pesticides on long use. In recent years there has been considerable force on consumers and farmers also to reduce or replace synthetic pesticides in agriculture to overcome with all these hazards. We are in need of effective tools to fight against pests, although we have a rich source of plants that could be treated as botanical pesticides.

Botanical pesticides also called green or organic pesticides are used widely until the 1940s. Botanical pesticides are alternative to synthetic pesticides, pests and pathogens are unable to developed resistance against these pesticides. Pyrethrum (*Chrysanthemum* sp.) [40], rotenone (*Derris* Sp.) [41], azadirachtin (*Azadirachta indica*) [42], and nicotine (*Tobbaco*) [43], quassin (*Picrasma excelsa*) [44], veratridine and cevadine (Cucumis sativa and Lactuca sativa) [45] are main compounds that were isolated from plants and used as pesticides from many years. Some other allelochemicals as Thymol (*Thymus vulgaris*), ajugarin I (*Ajuga remota*), abietic acid (*Pinus* sp.) and canavanine (Leguminaceae sp.) are also includes in most potent botanical pesticides [46]. Piperine is the major constituent of *Piper nigrum* (Piperaceae) has toxicity against several insects and repellent to adult corn earworms *Heliothis zea* [47]. 2-Tridecanone is a naturally occurring insecticide from the ***wild tomato*** species *Lycopersicon hirsutum f. glabratum* provides them resistant to attack by a number of insects, pests of the cultivated tomato, it is found in the tips of in glandular trichomes which around on the foliage [48] (Fig. **3**). Plants like *Albizia labbeck*, *Annona squamosa*, *Butea monosperma*, *Ficus indica*, *Madhuca indica* and *Azadirachta indica* used as a pesticide since ancient time. More than hundred insects and belongs to different orders can be easily controlled by using plant species [49]. Keeping in view all the facts, in present study we address the impact of extract, fractions and compounds of selected locally available three plants *Boenninghausenia albiflora*, *Skimmia anquetillia* and *Glycosmis arborea* from Rutaceae family with four plants of Verbenaceae family *Vitex negundo*, *Premna barbata*, *Callicarpa arborea* and *Clerodendron viscosum* to test their activities against pests, insects and micro-organisms.

3. PLANTS OF RUTACEAE AND VERBENACEAE FAMILY AS PESTICIDAL AND INSECTICIDAL AGENTS

Rutaceae is a wide class of plants it contains 160 genera and 1,700 species spread throughout the world. Plants of Rutaceae family are earlier reported for their insecticidal and pesticidal, antimicrobial and other biological activities [48]. This family belongs to citrus plants that have a variety of terpenes, alkaloids and flavonoids. Terpenes like limonoids are most potent active against pests, insects as well as on micro-organisms [50-52]. Pyranocoumarins, furanocoumarins from *Stauranthus perforatus* (Rutaceae) caused significant inhibition of growth of *Amaranthus hypochondriacus* and *Echinochloa crus-galli* [53]. Quinolinone alkaloids have been detected with antifeedant and growth inhibitor activity [54, 55]. Alkaloids and terpenoids present in *Teclea afzelii* are reported for its antimicrobial action [56].

Verbenaceae family includes some 35 genera and 1, 200 species, mainly tropical plants are the members of this family. Plants of this family are well known for their activity against pests, insect and other microorganisms. They are rich source of terpnes, flavones, phenolic glycosides and sterols that responsible for their biological activities. *Cleodandrum viscosum* is reported for its repellent action on *Tribolium confusum* [57], *Vitex negundo* leaf extract shows larvicidal activity against Japanese encephalitis vector and various adult vector mosquitoes [58]. Terpenoids isolated from leaves of *Vitex negundo* possess antifeedant activity [59]. *Vitex peduncularis* [60], *Callicarpa erioclona Schau*., *Callicarpa farinosa* and *Sphonodesma friflora* [61] are reported for their antimicrobial activity. Essential oils of flowers and leaves of *Lippia turbinate*, *Lippia polystachya*, *Lippia multiflora* verbenaceae family contains a large number of terpenes, cause of their potent insecticidal, antifeedant and repellent properties [62, 63].

4. MATERIALS AND METHODS

Plant Material. Rutaceae family: Leaves of *B. albiflora* were collected from Nagdev region at a height of 2200 m from Uttaranchal, India in September, 2003. *S. anquetillia* (leaves) col-

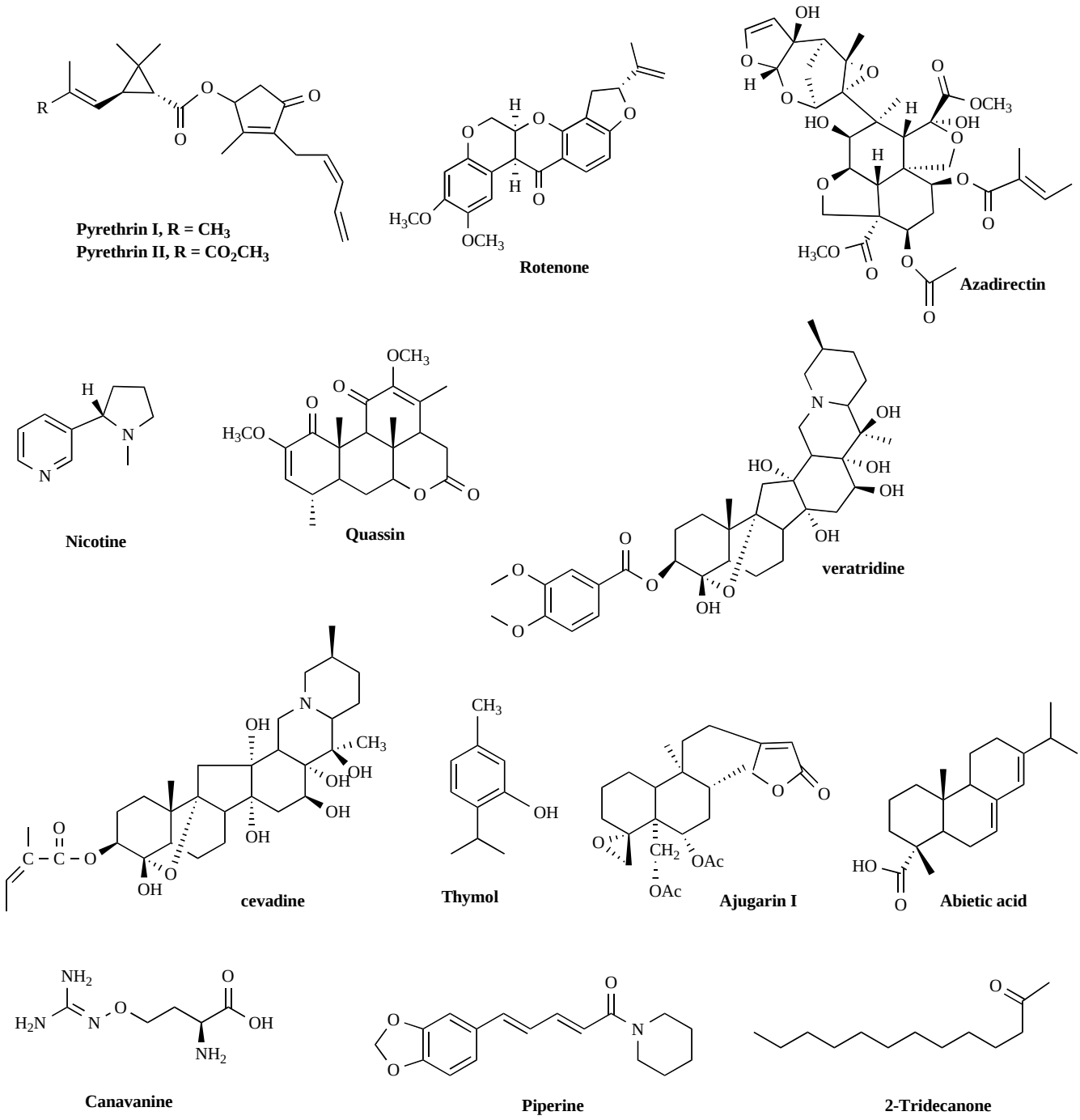

Fig. (3). Structures of some botanical pesticides.

lected from Tungnath Chopta, Uttaranchal, India at 3200 m height, *G. arborea* (leaves) were collected from Rajaji National Park, Haridwar, Uttaranchal, India. All plants were identified by the taxonomists of Department of Botany, HNB Garhwal University, India. A voucher sample has been deposited in herbarium of Department of Chemistry, HNB Garhwal University, India.

Verbenaceae family: two plants *P. barbata* (leaves) and *C. arborea* (leaves) were collected from Kotdwar, Uttarakhand, India in the month of August, 2008. The leaves of *C. viscosum* were collected from Rishikesh, Uttarakhand, India in the month of August, 2009. Collection of *V. negundo* (leaves) was made from Srinagar, Garhwal, India in August, 2008. Collected plans were identified by Botanical Survey of India, Dehradun, India and voucher specimen has been deposited there with voucher specimen numbers BSD Accession No. 112669, BSD Accession No. 112670, BSD Accession No. 112672 and BSD Accession No. 112671 for each plant respectively.

Extraction and Isolation. Shed dried leaves (4 kg each) of *B. albiflora*, *S. anquetillia* and *G. arborea* were individually extracted with 95% ethanol (EtOH) (four times) combined extracts of each plant were separately dried at 45 ^{o}C under reduced pressure . Dried EtOH extracts of *B. albiflora* and *S. anquetillia* were fractionated with n-hexane, chloroform and methanol however EtOH extract of *G. arborea* was fractionated with n-hexane and n-butanol (n-BuOH). n-BuOH fraction of *G. arborea* was further fractionated

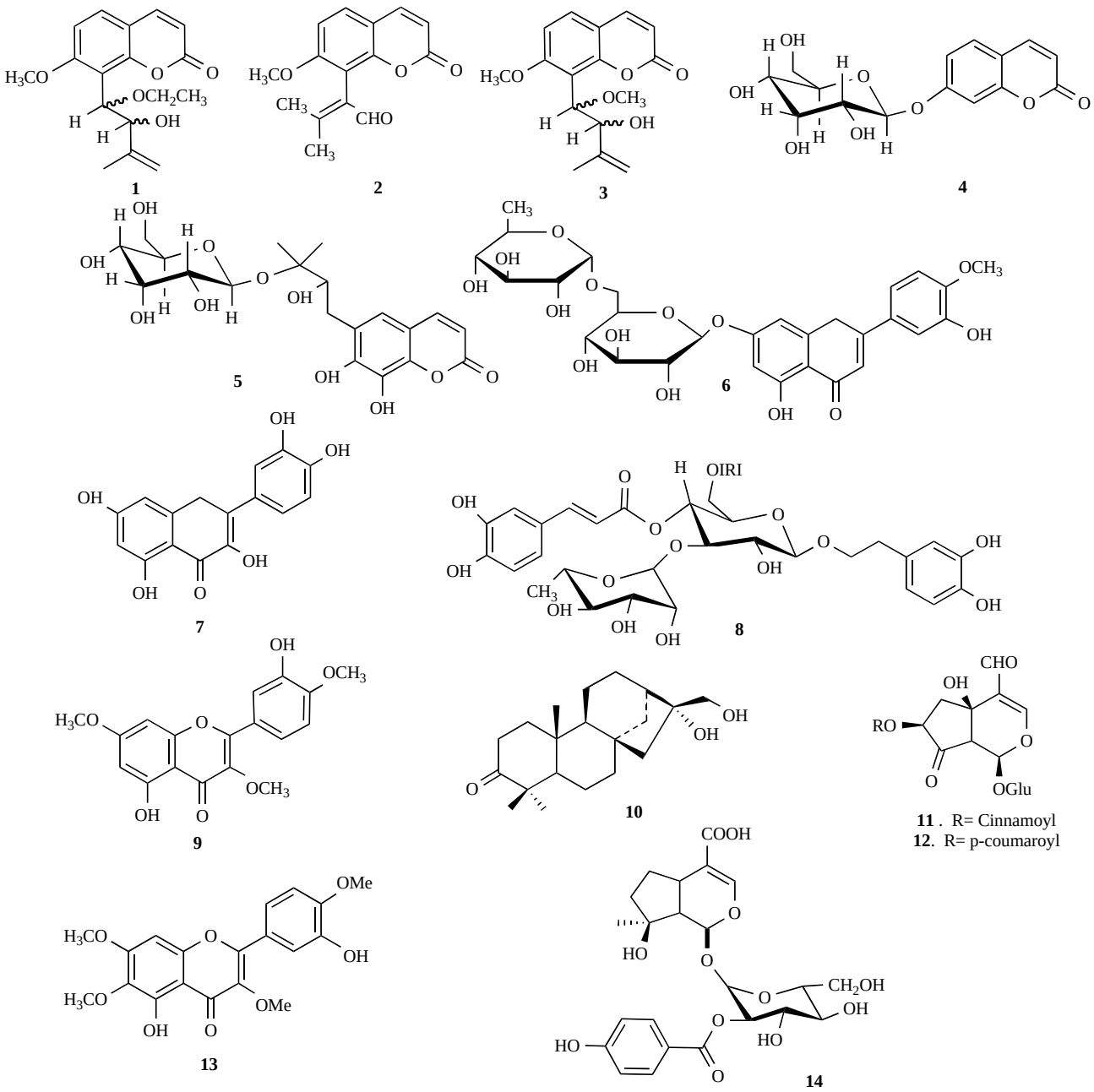

Fig. (4). Compounds isolated from leaves of selected plants from Rutaceae and Verbenaceae family.

with chloroform ($CHCl_3$), ethyl acetate (EtOAc) and methanol (MeOH).

For isolation of compounds MeOH fraction of *B. albiflora* (42.6 g) was subjected to column chromatography over silica gel 100-200 mesh by elution with $CHCl_3$: MeOH of increasing polarity to furnish twelve fractions which were pooled on the basis of same TLC pattern to make seven (F1-F7) fractions. Further column chromatographic purification of fraction F5 was led to the isolation of three active compounds, murraxocin (**1**) [64] (110 mg) in hexane: EtOAc, 70: 30, murralongin (**2**) [65] (30 mg) with hexane: EtOAc, 60: 40 and albiflorin-3 (**3**) [66] (42 mg) on elution of hexane: EtOAc, 60: 40. Column chromatographic separation of MeOH fraction of *S. anquetillia* (50.0 g) had been carried out on silica gel 100-200 mesh using $CHCl_3$: MeOH as mobile phase with elution of increasing polarity yields nine fractions of different polarity (F1-F9). Re-column chromatography of F2 leads to the isolation of two antibacterial compounds skimmin (**4**) [67] (82 mg) with $CHCl_3$: MeOH, 92: 8 and skimminan (**5**) [68] (98 mg) in $CHCl_3$: MeOH, 85: 15 eluate. MeOH fraction of *G. arborea* (32.7 g) was separated over silica gel 100-200 mesh with $CHCl_3$: MeOH, one active compound isolated as 7-*O*-(6-*O*-α-L-rahmnopyranosyl-β-D-glucopyranosyl)-3,3′,5,7-tetrahydroxy-4′-methoxyflavanone (**6**) [69] (46 mg) with $CHCl_3$: MeOH, 85: 15 (Fig. **4**).

Collected and shed dried leaves of *P. barbata*, *C. arborea*, *C. viscosum* and *V. negundo* (5 kg each) were separately extracted with 95% ethanol at room temperature. Combined extracts of each plant leaves were separately dried uder reduced pressure at 45 ^{o}C and fractionated with n-hexane, $CHCl_3$ and n-BuOH. n-BuOH fraction of *P. barbata* (58.23 g) was subjected to column chromatography over Silica gel 60-120, by using gradient elution of MeOH: $CHCl_3$ yielded ten fractions which were pooled on the basis of TLC analysis to make five fractions (F1-F5). Further flash column chro-

matographic purification of F4 on silica gel 230-400 mesh with the gradient elution of MeOH: $CHCl_3$ was led to the isolation of quercetin (**7**) [70] (40 mg) by MeOH: $CHCl_3$, 90: 10 and Premcoryoside (**8**) [71] (41.0 mg) with MeOH: $CHCl_3$ 95: 5. Hexane fraction of *C. arborea* (60.0 g) was subjected to extensive column chromatography on silica gel 100-200 mesh by elution with hexane: $CHCl_3$ mixtures of increasing polarity to furnish twelve fractions, on the basis on same TLC pattern fractions were pooled to make eight fractions (F1-F8). Re-column chromatography of F-3 afforded 5, 3'-dihydroxy-3, 7, 4'-trimethoxyflavone (**9**) [72] (39.0 mg) with elution of 90: 10 $CHCl_3$: hexane, calliterpenone (**10**) [73] (44.0 mg) isolated from $CHCl_3$ elution. Tannin free n-BuOH fraction of *C. viscosum* was chromatographed over silica gel 100-200 mesh by $CHCl_3$: MeOH mixtures of increasing polarity, 7-β-cinnamoyloxyugandoside (**11**) (54.0 mg) and 7-β- coumarroloxyugandoside (**12**) [74] (42 mg) both were isolated with 20: 80 MeOH: $CHCl_3$ eluate from this fraction. n-BuOH fraction of *V. negundo* (56.2 g) was subjected to column chromatography over silica gel 100-200 mesh, eluted with EtOAc: MeOH mixtures of increasing polarity this was furnished nine fractions, on the basis of TLC analysis the fractions were mixed to make six fractions (F1-F6), further chromatographic purification of F-4 over silica gel 230-400 mesh was led to isolation of vitexicarpin (**13**) [75] (60 mg) and 2′-p-hydroxybenzoyl mussaenosidic acid (**14**) [76] (57 mg) with EtOAc: MeOH, 80: 20 and 90: 10 respectively (Fig. **4**). All isolated compounds were characterized by the study of their UV, IR, ^{1}HNMR, ^{13}CNMR and mass spectroscopy data. Known compounds were identified by the comparison of their spectroscopy data from reported literature.

5. GENERAL EXPERIMENTAL PROCEDURES

The UV spectrums were recorded in Beckman 64 spectrophotometer. NMR spectrums were held on Bruker AVANCE 500 at 500 MHz for ^{1}H and for 125 MHz ^{13}C. Silica gel 100-200 mesh, (Qualigen) was used for column chromatography and silica gel G (Merck) used for TLC.

Insect antifeedant assay. The insecticidal assay was carried out on major pest of shisham *Plecoptera reflexa*, the popular defoliator *Clostera cupreta* and bamboo leaf roller *Crypsiptya coclesalis*. Tests were carried out at 20-26 ^{0}C in semidarkness. Leaf discs of young shisham plants were sprayed with an aqueous emulsion containing the plant extracts, fractions of selected plants and their isolated compounds at different concentrations. After spray coating had dried, the discs were placed in Petri dishes and five third instar *Plecoptera reflexa*, *Clostera cupreta* or *Crypsiptya coclesalis* larvae were placed on the discs for three days. Six discs were used for each concentration. Evolution was made on a percentage mortality basis.

Pest antifeedant assay. The dual choice leaf disc method as performed. Field collected *Ricinus communis* (Castor oil plant) were cut to circular discs (180 cm^2) with the median vein as the marker between two equal halves. Extracts and compounds were dissolved in acetone and water, sprayed on the right half of circular leaf disc to have the concentration of 2.5 $\mu m/cm^2$ leaf area for extracts and 50 $\mu m/cm^2$ for compounds, left half of leaf as treated with one ml acetone as control. After drying each leaf disc was placed in a Petri dish 15 cm. diameter. Five freshly moulded third instar larvae of *Spodoptera litura* from laboratory culture were placed in center of and left to feed for 24 hrs. Five replicates were maintained for each extract and compound. After 24 hrs the larvae were removed and unfed area in treated and control halves were measured by using ΔT area measurement meter. Percent Feeding Index (PFI) was calculated as:

$$PFI = * 100 \quad \frac{\% \text{ Area fed in treated}}{(\% \text{ Area fed in treated} + \% \text{ area fed in control})}$$

In case of growth inhibitors, Percent growth reduction is calculated as.

$$\% \text{ Growth Reduction} = 100 - \frac{\text{Treatment weight}}{\text{Control weight}} * 100$$

Antimicrobial assay. Three prominent plant pathogens, *Agrobacterium tumifaciens* (MTCC 609), *Pseudomonas syringae* (MTCC 1604) and *Pactobacterium carotovorum* (MTCC 1428) were procured from Microbial Type Culture Collection Institute of Microbial Technology (MTCC, IMTECH), Chandigarh, India). *P. syringae* were grown in nutrient agar (HiMEDIA) and *P. carotovorum* in Trypticase Soy Agar media (HiMEDIA). Disc diffusion method [77] was used to determine the zone of inhibition. Bacterial pathogens at their active log phase were spread over media plates. Subsequently the sterile discs of 5 mm diameter of filter paper were prepared. From a stock solution of 6 mg/ml, 200 and 500 µg of plant extracts and isolated compounds were applied to discs. The discs were air dried and then placed on a lawn of the test organism. The plates were incubated at 28 ^{0}C for 24 hrs. DMSO and MeOH were used as control. The zones of inhibition were observed around each disc.

6. RESULTS

Extracts, fractions and isolated compounds of all selected plants were tested for their antifeedant, insecticidal and antimicrobial activities against *Plecoptera reflexa*, *Clostera cupreta*, *Crypsiptya coclesalis* insects, *Spodoptera litura* pest, with three plant patho-

Table 1. Antifeedant Activity of Investigated Plants of Rutaceae Family Against *S. litura*

Plant	Particular	Percent Feeding Index (Mean±SD)
B. albiflora	Hexane fraction	44.34 ± 6.12
	$CHCl_3$ fraction	NS
	MeOH fraction	14.02 ± 11.05
2	(pure compound)	50.24 ± 1.08
3	(pure compound)	43.24 ± 2.41
S. anquetillia	Hexane fraction	35.23 ± 2.87
	$CHCl_3$ fraction	NS
	MeOH fraction	49.10 ± 4.17
G. arborea	Hexane fraction	46.71 ± 4.07
	$CHCl_3$ fraction	NS
	MeOH fraction	50.21 ±5.21

Values are expressed as mean ± SD.

Table 2. Insecticidal Activity of Investigated Plants of Rutaceae Family

Plant Name	Particular	Mortality (%)		
		1	2	3
B. albiflora	EtOH extract	70.0	75.0	-
1	Pure compound	80.0	85.0	-
S. anquetillia	EtOH extract	55.0	50.0	-
G. arborea	EtOH extract	-	-	-

(1) C. cupreta, (2) P. reflexa, (3) C. coclesalis

Table 3. Antibacterial Action of Investigated Plants of Rutaceae Family

Plant Name	Particular	Conc. µg/disc	Zone of Inhibition (mm)		
			1	2	3
B. albiflora	EtOH extract	500	-	-	-
S. anquetillia	Hex. fraction	500	-	-	-
	$CHCl_3$ fraction	500	-	-	-
	MeOH fraction	500	15	07	10
		200	12	08	09
4	Pure compound	500	08	-	-
		200	07	-	-
5	Pure compound	500	12	10	10
		200	09	09	07
G. arborea	EtOH extract	500	-	-	-
	Hex. fraction	500	-	-	-
	$CHCl_3$ fraction	500	-	-	-
	MeOH fraction	500	16	06	18
		200	12	-	14
6	Pure compound	500	11	-	-
		200	09	-	-

(1) A. tumifaciens, (2) P. syringae, (3) P. carotovorum

Table 4. Growth Inhibitor Activity of Investigated Verbenaceae Plants Against *S. litura* Pest

Plant Name	Particular	Mortility (%)	Growth red. (%)
P. barbata	Hex. fraction	13.00	26.01
	$CHCl_3$ fraction	25.00	43.93
	BuOH fraction	11.00	23.69
C. arborea	Hex. fraction	50.00	93.19
	$CHCl_3$ fraction	30.00	89.50
	BuOH fraction	NS	NS
C. viscosum	Hex. fraction	83.33	94.55
	$CHCl_3$ fraction	NS	12.73
	BuOH fraction	NS	NS

gens as *Agrobacterium tumifaciens*, *Pseudomonas syringae* and *Pactobacterium carotovorum*. The extracts and fractions of three plants of rutaceae family with two compounds (**2** and **3**) isolated from *B. albiflora* shows significant antifeedant activity against *S. litura* (Table **1**).

Ethanolic extracts of *B. albiflora* and *S. anquetillia* and one compound (compound **1)** from *B. albiflora* showed potent insecticidal property (Table **2**) but *G. arborea* of the family was found to inactive.

Testing results of antibacterial activity confirmed that plants of rutaceae family possess antibacterial properties, plant extracts, fractions and two compounds (**4** and **5**) from *S. anquetillia* with one compound (compound **6)** isolated from *G. arborea* were identified active against selected plant pathogens while *B. albiflora* was found to inactive (Table **3**). Insecticidal action of *B. albiflora* and compound **1** were also reported in our previous communication [64]. Plants of verbenaceae family investigated by us were not found active against insects and micro-organisms, but possess much better growth inhibitor activity against *S. litura* pest.

The results demonstrated that $CHCl_3$ fraction of *P. barbata* showed 25.0% mortality.Hexane fraction of *C. arborea* showed 50% mortality. Again hexane fraction of *C. viscosum* found to be active against *S. litura* with 83.33% mortality. 20% mortality showed in hexane fraction of *V. negundo* in comparison with 0.005 % Triton X 100 was treated as control (Table **4**). These results suggested that *C. arborea* and *C. viscosum* are most potent growth inhibitors while *P. barbata* and *V. negundo* were showed moderate

activity against *S. litura*. Compounds isolated from these plants were not showed growth inhibitor activity.

7. CONCLUSION

Natural pest controllers like botanical pesticides are safer to the user and environment because they break down into harmless compounds within hours or days in the presence of sunlight. Botanical pesticides are considered as plant protection agents. The agriculture food products that are produced in ecological farms by using natural fertilizers along with natural pest controllers are safe for human health and our surroundings. In this connection our study demonstrated the impact of plants of rutaceae and verbenaceae family on insects, pests and micro-organisms. *B. albiflora* and *S. anquetillia* (rutaceae) possess good insecticidal property. Ethanolic extract, fractions and compounds from *B. albiflora* and *S. anquetillia* were found to active against selected plant pathogens but only MeOH fraction of *G. arborea* shoed activity against pathogens, all antibacterial activities were carried out at a comparative concentrations of 500 and 200 μg/ disc. In addition all investigated plants of rutaceae and verbenaceae family were found to active against *S. litura* pest. Hexane fractions of *C. arborea* and *C. viscosum* (verbenaceae) were found as fine growth inhibitor with 50.00 % and 83.33 % mortality. Further research is required in this area to find out another potent alternative of synthetic pesticides.

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CONFLICT OF INTEREST

None declared.

REFERENCES

[1] Isman, M.B. Botanical insecticides, deterrents, and repellents inmodern agriculture and an increasingly regulated world. *Annu. Rev. Entomol*., **2006**. *51*, 45–66.

[2] Sanborn, M.; Kerr, K.J.; Sanin, L.H.; Cole, D.C.; Bassil, K.L.; Vakil, C. Non-cancer health effects of pesticides: systematic review and implications for family doctors. *Can. Fam. Physician*, **2007**, *53* (10), 1712–1720.

[3] Jurewicz, J; Hanke, W. Prenatal and childhood exposure to pesticides and neurobehavioral development: review of epidemiological studies. *Int. J. Occup. Med. Environ. Health*, **2008**, *21* (2), 121–132.

[4] Krieger, R.I. *Handbook of Pesticide Toxicology: Principles*, Vol. *1*, 2nd ed.; Academic Press: New York, **2001**.

[5] DeCanio, S.J.; Norman, C.S. Economics of the 'Critical use' of Methyl Bromide under the Montreal Protocol. *Contemporary Econ. Pol.* **2005**, *23* (3), 376–393.

[6] Alkofahi, A.; Rupprecht, J.K.; Anderson, J.E.; Mclaughlin, J.L.; Mikolajczak, K.L.; Scott, B.A. *In: Insecticides of plant origin*; T. Arnason; B.J.R. Philogene, and P. Morand., Eds; ACS Symposium Series 387. American Chemical Society: Washington DC, **1989**; pp. 23-43.

[7] Heal, R.; Rogers, R.T.; Wallaceyo, S. A survey of plants for insecticidal activity. *Lioydid*, **1950**, *13* (2), 889-162.

[8] Simmonds, M.S.J.; Evans H.C.; Blaney, W.M. *In:Pest Management and the Environment in 2000* AASA; A. Kadir; H.S. Barlow, Eds; CAB International: Wallingford, **1992**; pp. 127-164.

[9] Connolly, J.D. *In: Chemistry and Chemical Taxonomy of Rutales*; P.G. Waterman, and M.F. Grunden, Eds; Academic Press: London, **1983**; pp. 175-213.

[10] Feng, R. and Isman, M.B. Selection for resistance to azadirachtin in green peach aphid. *Experientia*, **1995**, *51*, 831-833.

[11] Isman, M.B. *In: Methods of Biotechnology*, Biopesticides: Use and Delivery; F.R. Hall and J.J. Mann, Eds.; Humana Press Inc.: Totowa, NJ, **1997**, Vol. *5*, pp. 139-153.

[12] Frazier, J. L. The perception of plant allelochemicals that inhibit feeding. *In: Molecular Aspects of Insect-Plant Associations*, L.B. Brattsten and S. Ahmad, Eds; Plenum Press: New York, **1986**; pp. 1–42.

[13] Kuhnau, J. The flavonoids: A class of semi essential food components: their role in human nutrition. *World Res. Nut. Diet*, **1976**, *24*, 117-191.

[14] Georgina, N.; Napal, D.; Carpinella, M.C.; Palacios, S.M. Antifeedant activity of ethanolic extract from *Flourensia oolepis* and isolation of pinocembrin as its active principle compound. *Bioresource Technol.*, **2009**, *100* (14), 3669-3673.

[15] Xu, D.; Huang, Z.; Cen, Y.-J.; Chen, Y.; Freed, S.; Hu, X.-G. Antifeedant activities of secondary metabolites from *Ajuga nipponensis* against adult of striped flea beetles, *Phyllotreta striolata*. *J. Pest. Sci.*, **2009**, *82* (2), 195-202.

[16] Addor, R.W. *In: Agrochemicals from natural products*; C.R.A. Godfrey, Ed.; Marcel Dakker Inc.: New York, **1994**, pp. 1-62.

[17] Masanori, M.; Kumiko, T.; Sachiko, N.; Takayoshi, O.;Ayako, N.; Koichiro, K. Insect antifeedant activity of flavones and choromones against *Spodoptera litura*. *J. Agric. Food Chem*., **2003**, *51*, 389-393.

[28] Langenheim, J.H. Higher plant terpenoids: a phytocentric overview of their ecological roles. *J. Chem. Ecol*., **1994**, *20*, 1223–1280.

[19] Katsiotis, S.T.; Langezaal, C.R.; Scheffer, J.J.C. Composition of the essential oils from leaves of various *Humulus lupulus* L. cultivars. *Flavour Fragr. J*., **1990**, *5*, 97-100.

[20] Latrasse, A.; Seamon, E.; Le Quere, J.L. Composition and major odorous compounds of the essential oil of *Bifora radians*, an aldehydeproducing weed. *J. High Resolut. Chromatogr*., **1991**, *14*, 549-553.

[21] Merrit, A.T. and Ley, S.V. Clerodane diterpenoids. *Nat. Prod. Rep*., **1992**, *9*, 243-287.

[22] Anderson, J.C.; Blaney, W.M.; Cole M.D.; Fellows, L.L.; Sheppaed R.N.; Simmonds, M.S.J. Structure of two new clerodane diterpeniods potent insects and antifeedants from *Scutellaria woronowii* (Juz): jodrellin A &B. *Tetrahedron Lett*., **1989**, 4737-4740.

[23] Harwood, S.H.; Moldenke, A.F.; Berry, *R.E.* Toxicity of peppermint monoterpenes to the variegated cutworm (Lepidoptera: Noctuidae). *J. Agric. Food Chem*., **1990**, *38*, 1406–1411.

[24] Soderlund, D.M. *In: Pyrethrum Flowers: Production, Chemistry, Toxicology, and Uses*; J.E. Casida, and G.B. Quistad, Eds.; Oxford University Press: New York, **1995**, pp. 297–233.

[25] Núñez, M.J.; Guadaño, A.; Jiménez, I.A.; Ravelo, A.G.; Coloma, A. G.; Bazzocchi, I.L. Insecticidal Sesquiterpene Pyridine Alkaloids from *Maytenus chiapensis*. *J. Nat. Prod.*, **2004**, *67* (1), 14–18.

[26] Cornelius, W.W.; Akeng'a, T.; Obeiro, G.O.;Lutta, K.P. Antifeedant activities of Erythria alkaloids from *Erythrina latissima* against *Spodoptera littoralis* (Lapidoptera noctuiidae). *Rec. Nat. Prod*., **2009**, *3* (2), 96-103.

[27] Tang, C.P.; Chen, T.; Velten, R.; Jeschke, P.; Kintscher, U.E.; Geibel, S.; Ye, Y. Alkaloids from stems and leaves of *stemona japonica* and their insecticidal activities. *J. Nat. Prod*., **2008**, *71* (1), 112–116.

[28] Tringali, C.; Spatafora, C.; Calì, V.; Simmonds, M.S.J. Antifeedant constituents from *Fagara macrophylla*. *Fitoterapia*, **2001**, *72* (5), 538-543.

[29] Nissanka, A.P.K; Karunaratne, V.; Bandara, B.M.R.; Kumar, V.; Tsutomu, N.; Nishi, M.; Inada, A; Tillakratne, L.M.V.; Wijesundara, D.S.A; Gunatilaka, A.A.L. Antimicrobial alkaloids from *Zanthoxylum tetraspermum* and *caudatum*. *Phytochemistry*, **2001**, *56*, 857-861.

[30] Gerson, H. Fungi toxicity of 1-4, naphthaquinones to Candida albicans and Trychophyton mentagrophytes. *Can. J. Microbiol*., **1975**, *21*, 197-205.

[31] Schuerch, A.R.; Wehrli, W. Lapachone, an inhibitor of oncornavirus reverse transcriptase and eukaryotic DNA polymerase inhibitory effect, thiol dependence and specifity. *Eur. J. Biochem*., **1978**, *84*, 197-205.

[32] Wagner, H.; Kreher, B.; Lotter, H.; Hamburger, M.O.; Cordell, G.A. Structure determination of new isomeric naphthol [2,3,-b]furan 4,9-dionesfrom Tabebuia avellanedae by selective INEPT techniques. *Helv. Chim. Acta*, **1989**, *72*, 659-667.

[33] Avilla, J.; Teixidò, A.; Velázquez, C.; Alvarenga, N.; Ferro, E.; Canela, R. Insecticidal Activity of *Maytenus* Species (Celastraceae) Nortriterpene Quinone Methides against Codling Moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). *J. Agric. Food Chem.*, **2000**, *48* (1), 88–92.

[34] Rodriguez, E. *In: Plant Resistance to Insects*; Paul A. Hedin, Eds.; ACS Publications: Washington, DC, **1983**; Vol. *208*, pp. 291–302.

[35] Banerjee, S.; Magdum, S. *In: Perspectives in animal ecology and reproduction*; V. K. Gupta and A. K. Verma, Eds.; Daya Publishing House: Delhi, India, **2007**, Vol. *4*, pp. 341-375.

[36] *Bowers W.S.*; Fales, H.M.; Thompson, M.J., and Uebel, E.C. Juvenile hormone: Identification of an active compound from balsam fir. **Science**, **1966**, **154**, ***1020***-1022.

[37] Henrick, C.A. Juvenoids. *In: Agrochemicals from Natural Products*; C.R.A. Godfrey, Ed.; Marcel Dakker, Inc.: New York., **1994**, II series, pp. 147-341.

[38] Bowers W.S.; Ohta, T.; Cleere, J.S.; Marsella, P.A**.** Discovery of insect antijuvenile hormones in plants? 2U. *Science*, **1976**, *193* (4253), 542 – 547.

[39] NRI. *A synopsis of integrated pest managemen in developing countries in the tropics*. Natural Resources Institute, Chatham, UK, **1992**.

[40] Katsuda, Y. Development of and future prospects for pyrethroid chemistry. *Pestic. Sci*., **1999**, *55*, 775-782.

[41] Fang, N.; Casida, J.C. resin insecticide: identification and biological activity of 29 rotenoid constituents. *J. Agric. Food Chem.*, **1999**, *47* (5), 2130–2136.

[42] Mandal, R.K.; Bhattacharya, A.K. Azadirachtin, the most potent neem derivative effect on *Spilosoma obliqua*. *Indian J. Ent*., **2003**, *65* (2), 170-176.

[43] Schmelz, I. *In: Naturally Occurring Insecticides*; M. Jacobson; D.G. Crosby; M. Dekker, Eds.; Academic ***Press***: New York, **1971**, pp. 99-136.

[44] Scragg, A.H.; Allan, E.J. *In: Medicinal and aromatic plants*; Y.P.S. Bajaj Ed.; Springer-Verlag: New York, **1994**, Vol. *6*, pp. 316-326.

[45] Zang, X.; Fukuda, E.K.; Rosen, J.D. Method for the Determination of Veratridine and Cevadine, Major Components of the Natural Insecticide Sa-

badilla, in Lettuce and Cucumbers. *J. Agric. Food Chem*., **1997**, *45* (5), 1758–1761.

[46] Isman, M. Insect Antifeedants. *Pesticide Outlook*, **2002**, 152-157.

[47] Paula, V.F.D.; Barbosa, L.C.D.A.; Demuner, A.J.; Veloso, D.P.; Picanco, M.C. Synthesis and insecticidal activity of new amide derivatives of piperine. *Pest Manage. Sci*., **2000**, *56*, 168-174.

[48] Dimck, M.B.; Kennedy, G.G. Willium, W.G. toxicity studies of analogs of 2-tridecanone, a naturally occurring toxicant from a wild tomato. J. Chem. Ecol., 1982, 8 (5), 837-842.

[49] Gahukar, R.T. *Neem in Plant Protection*, Ist ed.; Agri-Horticulture Publishing Company, Nagpur: India, **1995**.

[50] Sharker, S.M.; Shahid, I.J.; Hasanuzzaman, M. Antinociceptive and bioactivity of leaves of *Murraya Peniculata* (L.) Jack, Rutaceae. *Rev. Bras. Farmacogn.*, **2009**, *19* (3), 746-748.

[51] Huang, R.C.; Tadera, K.; Yagi, F. Limonoids from the *Melia azedarach*. *Phytochemistry*, **1996**, *43* (5), 581-583.

[52] Kumar, C.S.S.R.; Srinivas, M.; Yakkundi, S. Limonoids from the Seeds of *Azadirachta indica*. *Phytochemistry*, **1996**, *42* (2), 451-455.

[53] Anaya, A.L.; Rubalcava, M.M.; Ortega, R.C.; Santana, C.G.; Monterrubio, P.N.S.; Bautista, B. E.H.; Mata, R. Allelochemicals from *Stauranthus perforatus*, a Rutaceous tree of the Yucatan Peninsula, Mexico. *Phytochemistry*, **2005**, *66* (4), 487-494.

[54] Riov, J.; Monlise, S.P.; Kahan, R.S. Ethylene-controlled Induction of Phenylalanine Ammonia-lyase in Citrus Fruit Peel. *Plant Physiology*, **1969**, *44*, 631-635.

[55] Miller, J.S. and Feeny, P. Effects of benzylisoquinolene alkaloids on larvae of *Polyphagous lapidoptera*. *Oecologia* (Berlin), **1983**, *58*, 332-339.

[56] Kuete,V.; Wansi, J.D.; Mbaveng, A.T.; Sop, M.M.K.; Tadjong, A.T.; Beng V.P.; Etoa, F.-X.; Wandji, J.; Marion Meyer, J.J.; Lall, N. Antimicrobial activity of the methanolic extract and compounds from *Teclea Afzelii* (Rutaceae). *S. Afr. J. Bot*., **2008**, *74* (4), 572-576.

[57] Husain, M.M. and Hasan, R. Repellency of indigenous plant, Bhant (*Cleodandrum viscosum Vent.*) on *Tribolium Confusum. Bangladesh J. Sci. Ind. Res.*, **2008**, *43* (2), 267-272.

[58] Karunamoorthi, K.; Ramanujam, S.; Rathinasamy, R. Evaluation of leaf extracts of *Vitex negundo L*. (Family: Verbenaceae) against larvae of *Culex tritaeniorhynchus* and repellent activity on adult vector mosquitoes. *Parasitol. Res*., **2008**, *103*, 545-550.

[59] Chandramu, C.; Rao, D.M.; David, G.L.K.; Reddy V.D. Isolation, characterization and biological activity of betulinic acid and ursolic acid from *Vitex negundo* L. *Phytother. Res*., **2003**, *17* (2), 129-134.

[60] Prusti, A.; Mishra, S.R.; Sahoo, S.; Mishra, S.K. Antibacterial Activity of Some Indian Medicinal Plants. *Ethnobotanical Leaflets*, **2008**, *12*, 227-230.

[61] Chung, P.Y.; Chung, L.Y.; Ngeow, Y.F.; Goh, S.H.; Imiyabir, Z. Antimicrobial Activities of Malaysian Plant Species. *Pharm. Biol*., **2004**, *42* (4-5), 292-300.

[62] Gleiser, R.M. and Zygadlo, J.A. Insecticidal properties of essential oils from *Lippia turbinata* and *Lippia polystachya* (Verbenaceae) against *Culex quinquefasciatus* (Diptera: Culicidae. *Parasitol. Res*., **2007**, *101* (5), 1349-1354.

[63] Owolabi, M.S.; Ogundajo, A.; Lajide, L.; Oladimeji, M.O.; Setzer, W.M.; Palazzo, M.C. Chemical composition of the essential oil of *Lippia multiflora* Moldenke from Nigeria. *Rec. Nat. Prod*., **2009**, *3* (4), 170-177.

[64] Sharma, R.; Negi, D.S.; Shiu, W.K.P.; Gibbons, S. Characterization of an insecticidal coumarin from *Boenninghausenia albiflora*. *Phytother. Res.*, **2006**, *20*, 607-609.

[65] Joshi, P.C.; Mandal, S.; Das, P.C. Jayantinin, a dimeric coumarin from *Boenninghausenia albiflora Phytochemistry*, **1989**, *28* (4), 1281-1283.

[66] Joshi,P.C.; Mandal, S.; Das, P. C.; Chatterjee A. Two minor coumarins of *Boenninghausenia albiflora Phytochemistry*, **1993**, *32* (2), 481-483.

[67] Khalil, A.T.; Chang, F.R.; Lee, Y.H.; Chen,C.Y.; Liaw, C.C.; Ramesh P.; Yuan, S.S. F.; Wu Y.C. Chemical constituents from the *Hydrangea chinensis. Arch*. *Pharmacal Res*., **2003**, *26* (1), 15-20.

[68] Sharma, R.K.; Negi D.S.; Gibbons S.; Otsuka H. Chemical and Antibacterial Constituents of *Skimmia anquetillia, Planta Med*. **2008**, *74* (2), 175-177.

[69] Kuroyanagi, M.; Ishii, H.; Kawahara, N.; Sugimoto, H.; Yamada, H.; Okihara, K.; Shirota, O. Flavonoid glycosides and limonoids from *Citrus* molasses. *J. Nat. Med*., **2007**, *62* (1), 107-111.

[70] Lallemand, J.Y.; Duteil, M. ^{13}C n.m.r. spectra of quercetin and rutin. *Org. Magn. Reson*., **1977**, *9* (3), 179–180.

[71] Otsuka, H.; Watanabe, E.; Yuasa, K.; Ogimi, C.; Takaushi, A.; Takeda, Y. A verbascoside iridoid glucoside conjugate from Premna corymbosa, var. obtusifolia, *Phytochemistry*, **1993**, *32* (4), 983-986.

[72] Mohammad Ali, N.A.; Rahmani, M.; Shaari, K.; Ismail, H. B.M.; Sukari, M.A.; Ali, A.M.; Kulip, J. Chemical constituents of leaves and barks of *Melicope Hookeri* T.G. Hartley, *Pertanika J. Sci. Technol*., **2006**, *14* (1&2), 75-80.

[73] Fujita,E.; Ochiai, M.; Ichida, I.; Chatterjee, A.; Desmukh S.K. Confirmation of the structure of calliterpenone, a diterpene from *Callicarpa macrophylla*. *Phytochemistry*, 1975, *14* (10), 2249-2251.

[74] Wei, X.M.; Zhu, Q.X.; Chen, J.C.; Cheng, D.L. Two new iridoid glucosides from Clerodendrum serratum, *Chin. Chem. Lett*., **2000**, *11* (5), 415-416.

[75] Yoshioka, T.; Inokuchi, T.; Fujioka, S.; Kimura, Y. Phenolic compounds and flavonoids as plant growth regulators from fruit and leaves of Vitex royundifolia, Z. Naturforsch, **2004**, *59 c*, 509-514.

[76] Sehgal, C.K.; Taneja, S.C.; Dhar, K.L.; Atal, C.K. 2'-p- Hydroxybenzoyl mussaenosidic acid, a new irridoid glucoside from *Vitex negundo*. *Phytochemistry*, **1982**, 21, 363-366.

[77] Salie, F.; Eagles, P.F.X.; Leng, H.M.J. Preliminary antimicrobial screening of four South African Asteraceae species *J. Ethanopharmacol*., **1996**, *52*, 27-33.

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