New possible insect growth regulators from *Catharanthus roseus*

Application of plant-derived insect growth regulator (IGR) substances is known to be safe for man and environment in the integrated pest management programme(s)[1]. So far, only azadirachtin – an IGR derived from Indian neem (*Azadirachta indica*) seed kernel is being commercialized and marketed for managing agricultural insect pests[2]. However, the substitute(s) of such naturally occurring insecticide prototypes are in great demand at a global level with a view to manage the insect resistance and safety to environment. Under the screening of developing natural plant products as pest control agents[3], we have reported himachalol and β-himachalene as natural insecticide molecule(s) from essential oil of *Cedrus deodara*[4,5]. In continuing the work on the isolation of IGRs from natural products of plant origin, we have found that *n*-hexane fraction of acetone extract from *Catharanthus roseus* leaf contains α-amyrin acetate[6] and oleanolic acid[7] as natural source of IGR against tobacco caterpillar (*Spodoptera litura* F.)[8] and gram pod borer (*Helicoverpa armigera* Hub.)[9] under bio-directed isolation of active principles.

The full-grown plant of periwinkle [*Catharanthus roseus* (Linn.) G. Don var. *rosea*] (pink flowered) was cultivated at the research farm of the Central Institute of Medicinal and Aromatic Plants, Lucknow, and harvested at reproductive stage. The leaves were collected, shade-dried and finely powdered. Air-dried and powdered leaves were extracted with MeOH; the combined extracts were concentrated and diluted with H_2O. The aqueous methanolic extract was then fractionated successively with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH. Removal of solvents from these extracts afforded the respective extracts. Secondly, the leaf was extracted with acetone and the extract was diluted with water. The aqueous extract was fractionated into *n*-hexane, EtOAc and EtOAc-insoluble fractions. The elimination of solvent afforded respective residues. Under the bio-directed isolation of active compounds, fractions were purified and identified.

Fresh and healthy larvae of *Spodoptera litura* Fab. and *Helicoverpa armigera* Hub. were collected from natural habitat of the CIMAP research farm, Lucknow. The larvae of *S. litura* were reared on fresh castor (*Ricinus communis*) leaves and *H. armigera* on semi-synthetic diet consisting of chickpea seed powder, yeast, vitamin mixture, anti-microbial, phagostimulants, fixing agent, etc., as described by Singh and Rembold[10]. The sixth instar fresh and healthy larvae of one age group were taken for the bio-assay. The larvae were maintained at a photo-period of 16 h light and 8 h dark cycle at 27 ± 1°C temperature and 60–70% relative humidity.

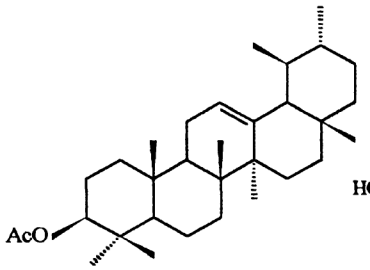
The insect larvae were anaesthetized with Et_2O. Each leaf extract of different solvents was dissolved in acetone at the rate of 2% concentration, separately and bio-assayed by topical application method using Arnold Hand Micro-applicator (M/s Burkard Manufacturing Co. Ltd, Rickmansworth Herts, England). Each fraction of the extract was dissolved at the rate of 2% in acetone and applied topically at the dose of 5 µl/6th instar larvae of *S. litura* and *H. armigera* Hub. The treatments were applied individually on each larva in batches of 40 test insects. The insects treated with acetone served as control. The azadirachtin-rich neem base commercial insecticide and a promising synthetic insecticide-dichlorvos were used as standard and tested at the concentration of 0.001% each. The observations were recorded on mortality and morphogenetic effects on the larvae, pupae and adults until the adult emergence. The IGR activity was recorded on the basis of both mortality and deformities in different stages of test insects and the per cent IGR activity calculated[11]. The corrected per cent IGR activity was calculated by the formula of Abbott[12].

Maximum IGR activity (84.21%) was observed from the topical application of acetone extract of *C. roseus* leaves against the larvae of *S. litura* compared to the extracts of methanol (36.84%), *n*-hexane (52.63%), chloroform (36.84%), EtOAc (63.51%), butanol (36.84%) and aqueous methanol (57.89%). The bioassay of acetone extract (68.42%) and the different fractions of *n*-hexane, EtOAc and EtOAc-insoluble fractions showed 52.63, 42.10 and 57.89% IGR activity against *S. litura* larvae, respectively. However, azadirachtin-rich insecticidal formulation and dichlorvos showed 84.21 and 78.94% of IGR activity over control under laboratory conditions, respectively.

Since acetone extract of *C. roseus* leaf also showed promising IGR activity (57.14%) against *H. armigera*, different fractions of acetone extract were also bio-assayed for their activity along with an azadirachtin-rich insecticidal formulation and dichlorvos. Among fractionated acetone extract of *n*-hexane, ethyl acetate and ethyl acetate-insoluble fractions results showed IGR activity as 67.85, 57.14 and 64.28% respectively against *H. armigera* larvae. The *n*-hexane fraction was found to contain active principles α-amyrin acetate (**I**) and oleanolic acid (**II**) (schemes 1 and 2), which were separated chromatographically and identified by spectral data. However, the ethyl acetate and ethyl acetate-insoluble fractions were not purified. Among *n*-hexane fraction of acetone extract, major compounds, the α-amyrin acetate (**I**) gave 35.71% IGR activity against *H. armigera*. The results showed that two-fold higher activity was found in the hexane fraction of acetone extract (containing α-amyrin acetate and oleanolic acid) compared to purified α-amyrin acetate against *H. armigera* larvae.

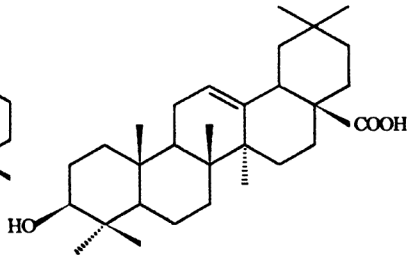
The demand for natural insecticides for controlling major insect pests of agriculture, health, and forestry is increasing continuously due to major demerits of the synthetic insecticides at the global level. The synthetic insecticides developed directly from petrochemicals possess major disadvantages like residue problem, development of resistance in insects, pest resurgence, secondary pest out-break, and destruction of beneficial insects that pose a great threat to man and environment[13]. So far, more than 2000 plant species have been identified which possess insecticidal properties, however, only a few of these plants have been used commercially due to one or other reasons to meet the growing worldwide demand for natural pesticides[14]. In this direction, we have screened a number of different plant extracts/essential oils against various insect pests in our laboratory and found that further detailed study can explore the possibility of *C. roseus* as source of new molecule(s) as natural insecticides for managing the serious field pests.

The insecticidal properties of *C. roseus* have been reported against *Amsacta*



(I)

Scheme 1. α-Amyrin acetate $C_{32}H_{52}O_2$, MW 468.



(II)

Scheme 2. Oleanolic acid $C_{30}H_{48}O_3$, MW 456.

moorei[15], *Dysdercus cingulatus*[16], *Pthroimaea operculella*[17], *Spodoptera littoralis*[18] and *S. litura*[19–26]. However, we found *n*-hexane fraction from acetone extract (containing α-amyrin acetate and oleonolic acid) as one of the natural sources of IGR against larvae of *S. litura* and *H. armigera* under bio-directed isolation of active principle(s) from *C. roseus* (Linn.) G. Don var. *rosea*. Our results indicate that α-amyrin acetate possess IGR activity (35.71%) against *H. armigera* larvae. The oleanolic acid obtained from *Panax quinguefolium* has been reported as insect feeding deterrence activity[27]. However, the biological activity of α-amyrin acetate against insects has not been reported so far, until the present report.

Indian neem (*Azadirachta indica* A. Juss.) based insecticides are presently commercialized to control field insect pests of agriculture. However, the short supply of raw material of neem fruits limits the production of insecticides required to meet the growing demand of botanical insecticide. The active insecticidal principle, azadirachtin of *A. indica*, is found in the neem seed kernels. The neem tree requires longer duration for its fruit formation, about 10–15 years, during their growth and development and its cultivation is restricted because of its perennial nature. Secondly, the major active insecticidal molecule (azadirachtin) of Indian neem cannot be easily synthesized economically on commercial scale due to its complex structure. However *C. roseus*, a medicinal plant being a major natural source of vinblastine and vincristine, is used as an anti-cancer drug. This is an annual field crop which can be easily grown in both tropical and temperate climates without the involvement of any specific agro-technology.

The IGR principle(s) obtained from *C. roseus* may be used as replacement of synthetic insecticides and substitute for existing botanical insecticides derived from neem (*Azadirachta indica*), pyrethrum (*Chrysanthemum cinerariaefolium*), derris (*Derris* spp), etc.[28]. Besides, repeated application of one kind of chemical formulation to control pests may provide a congenial environment for rapid development of insect resistance. Thereby, additional natural insecticides are continuously required to manage the development of resistance in insect pests.

Many workers have highlighted the importance of developing botanical insecticides from plants. It has been suggested that tremendous interest has been generated in recent years about the use of pesticidal plants, particularly those that can be harvested, formulated and used easily[29]. Similarly, two new insecticidal molecules (bufadienolide 1 & 2) have been isolated from the leaves of *Kalanchoe pinnata* by biodirected fractionation[30]. The fractions and active principles of *C. roseus* may also be bioassayed for its biological activities against other economic pests of agriculture. The hexane fraction of acetone extract (containingα-amyrin acetate) and/or prototypes of α-amyrin acetate could be used as active principle in developing new insecticides. It is also suggested that priority may be given to screen the annual plant species for insecticidal activity for the continuous supply of anti-insect substances for the raw materials over fruit trees.

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Hepatoprotective activity of *Sarcostemma brevistigma* against carbon tetrachloride-induced hepatic damage in rats

Liver diseases are a serious health problem. In the absence of reliable liver-protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. However, we do not have satisfactory remedy for serious liver disease; most of the herbal drugs speed up the natural healing process of liver. So the search for effective hepatoprotective drug continues.

Sarcostemma brevistigma Wight (family Asclepiadaceae) grows throughout India and other tropical regions of the world. It is found to be active as anti-rheumatic, anti-allergy, anti-emetic and branchodilator[1]. Phytochemical studies reveal the presence of bergenin, brevine, brevinine, sarcogenin, sarcobiose and flavonoids[2–4]. The present study was made to evaluate the effect of ethyl acetate extract of stem of *S. brevistigma* against CCl_4-induced hepatic damage in rats.

The stem of *S. brevistigma* was collected in June from the Kolli hills. The plant was authenticated by the Botany Department, Tamil Nadu Agricultural University, Coimbatore. A voucher specimen has been preserved in our laboratory. The plant was dried and powdered. The dried and powdered plant was extracted with ethyl acetate using soxhlet apparatus and concentrated *in-vacuo*. Approximately, 0.50 g of dried ethyl acetate extract was obtained from 10 g of dried stem material. The extract was suspended in 5% gum acacia and used for studying hepatoprotective activity.

Male albino rats weighing between 150 and 175 g were used as animal models. The rats were divided into four groups, each group consisting of six animals. Hepatoprotective activity of *S. brevistigma* was evaluated using CCl_4-induced model[5]. Group one was kept on normal diet and served as control, the second group received CCl_4 (1.25 ml/kg) by oral route, the third and fourth group received silymarin (100 mg/kg; po) and extract of *S. brevistigma* (650 mg/kg; po) respectively once daily, for seven days. On the seventh day, CCl_4 was given by oral route 30 min after the administration of silymarin and test drug. After 36 h of CCl_4 administration, blood was collected and serum separated was analysed for various biochemical parameters.

Biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT)[6], serum glutamic pyruvate transaminase (SGPT)[6], alkaline phosphatase[7], total bilirubin[8] and gamma glutamate transpeptidase (GGTP)[9] were analysed.

The liver was examined grossly, weighed and stored in formalin 10% and were processed for paraffin embedding using the standard microtechnique[10]. A section of the liver (5 µm) stained with alumhemotoxylin and eosin was observed microscopically for histopathological studies.

The results of biochemical parameters revealed the elevation of enzyme level in CCl_4-treated group, indicating that CCl_4 induces damage to the liver (Table 1). Liver tissue rich in both transaminase increased in patients with acute hepatic diseases, SGPT which is slightly elevated by cardiac necrosis is a more specific indicator of liver disease[11]. A significant reduction ($P < 0.001$) was observed in SGPT, SGOT, ALP, total bilirubin and

Table 1. Effect of ethyl acetate extract of *S. brevistigma* on CCl_4-treated rats

Design of treatment	Liver (wt/100 g body wt)	Dose (mg/kg)	SGPT U/L	SGOT U/L	ALP U/L	Total Bil (mg%)	GGTP U/L
Control	3.4 ± 0.10	–	131.5 ± 1.98	45.3 ± 0.80	160.6 ± 3.79	0.70 ± 0.03	123.0 ± 4.10
CCl_4	6.5 ± 0.28	1.25 ml/kg	217.3 ± 4.5	341.5 ± 3.8	388.6 ± 18.25	2.12 ± 0.01	251.0 ± 5.31
Silymarin + CCl_4	3.8 ± 0.26*	100	138.0 ± 2.17**	81.3 ± 9.10*	218.6 ± 5.47**	0.8 ± 0.07*	109.6 ± 5.20**
Ethyl acetate extract + CCl_4	4.1 ± 0.05*	650	115.3 ± 1.16*	67.0 ± 5.79*	292.6 ± 5.32*	0.83 ± 0.01*	133.3 ± 3.41**

$N = 6$ animals in each group.
*$P < 0.001$; **$P < 0.01$ when compared with control.
Values are expressed as mean ± SE.