Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(2): 300-306

Received: 10-01-2021
Accepted: 12-02-2021

N Revathi
PG and Research Department of Zoology, Sri Vasavi College, Erode, Tamil Nadu, India

Corresponding Author:
N Revathi
PG and Research Department of Zoology, Sri Vasavi College, Erode, Tamil Nadu, India

Efficacy of the medicinal plant, *Andrographis paniculata* (Burm. F.) Wall. against hadda beetle, *Henosepilachna vigintioctopunctata* (Fabricius) (Coleoptera: Coccinellidae) on bitter gourd

N Revathi

Abstract
The current global trend is towards consumption of food produced using safe and preferably natural plant protection products. Detection of hazardous chemical pesticide residues in foods and increased consumer awareness on food safety has resulted to ban of certain pesticides in agricultural production and plant-based pesticides are gaining popularity in organic agriculture. The disadvantages associated with the misuse and overuses of synthetic pesticides have stirred the need for alternative pest management options. Plants with bioactive compounds have been used to manage different crop pests and human infections with notable success. The importance of botanical pesticides is attributed to their efficacy, biodegradability, varied modes of action, low toxicity as well as availability of source materials. Active compounds sitosterol and stigmasterol isolated from *Andrographis paniculata* were evaluated for its antifeedant, larvicidal and adulticidal activity against *Henosepilachna vigintioctopunctata*. Isolated compounds stigmasterol and β-sitosterol were evaluated for their antifeedant activity at 250, 500, 750 and 1000 ppm concentrations against *H. vigintioctopunctata*. Stigmasterol at 1000 ppm exhibited a higher phagodeterrency of 67.83% than β-sitosterol (55.87%). Stigmasterol exhibited maximum larval mortality of 76.0% at 1000 ppm. Maximum adult mortality of 84.0% at 1000ppm was observed with stigmasterol. Apart from the known compound andrographolide, phytosterols isolated from *A. paniculata* also exhibited promising pest control activities.

Keywords: *Andrographis paniculata*, *Henosepilachna vigintioctopunctata*, antifeedant, larvicidal, adulticidal

1. Introduction
The use of synthetic pesticides in controlling agricultural insect pests has increased 12 fold in the last decade (Pimentel, 1997) [1]. Most synthetic insecticidal compounds fall within 4 main classes: organochlorines, organophosphates, carbamates and pyrethroids. Though synthetic pesticides have played a significant role in the protection of crops from the attack by insect pests, it has led to several problems such as environmental pollution, loss of biodiversity, health hazards to humans and animals, development of resistance in pests to pesticides, resurgence of target and non-target pests, destruction of beneficial organisms like parasitoids, predators, honeybees, pollinators, etc., and pesticide residues in food, fodder and feed (Jayaraj, 2007) [2].

Toxicity of synthetic insecticides is mainly restricted to neuro-muscular function. They require special safety procedures and equipments during production and application because of the exposure risks for humans, the environment and food. The use of organochlorine insecticides has been banned in developed countries (Erturk, 2006) [6]. It has been estimated that hardly 0.1% of the agrochemical used in crop protection reach the target pests and remaining 99.9% enter the environment and cause hazards to non-target organisms. Due to higher dose and repeated frequency of application, every year one million people suffer from pesticide poisoning (Bami, 1997) [4].

An alternative eco-friendly strategy for management of noxious insect pests has been searched to reduce harmful effects of synthetic chemical insecticides. Rampant use of synthetic pesticides has given rise to several short-term and long-term effects (Gupta, 2004). In recent years, crop protection based on botanicals to control pests has been recognized as a valuable tool in pest management (Kannaiyan, 2002) [6].

Botanicals are low in capital cost, easily biodegradable, less prone to pest resistance and eco-friendly. Developing a new generation of "green" pesticides from medicinal plants would be an effective alternative to hazardous synthetic chemical pesticides (Leatemia and Isman, 2004) [7]. The neem tree - Azadirachta indica has been known as the wonder tree for centuries in the Indian subcontinent. Pradhan *et al* (1962) have first demonstrated the antifeedant effect of neem kernel extracts against *Shistocerca gregaria*.

Andrographis paniculata is otherwise called Kalmegh (King of Bitters; Family Acanthaceae), native to Sri Lanka and India ((Nosalovaa *et al*., 2014) [8]. The plant *A. paniculata* is traditionally used as a medicine to treat different diseases in southern Asia, China and India (Govindarajan, 2011) [9]. *Momordica charantia*, the bittergourd is one of the most priced and widely cultivated plants in South Asia, especially India. A compound "charantin" present in the bittergourd is used in the treatment of diabetes to reduce blood sugar level (Palada and Chang, 2003) [10]. *Henosepilachna vigintioctopunctata*, a polyphagous coleopteran is a major pest of bittergourd and it brings crop loss up to 60%. Several studies reports that the plant to contain phytochemicals which have antibacterial, hypoglycemic, hypercholesterolemia, and adaptogenic properties (Shahid, 2011) [11]. The crude extract of *A. paniculata* shows larvicidal activity against many insects such as *Aedes aegypti* (Paul *et al*., 2020) [12], *Tribolium castaneum* (Baliyarsingh *et al*., 2020), and *Papilio demoleus* (Vattikonda, 2015) [14]. This study evaluated the larvicidal and pupicidal effects of *A. paniculata* on the survival on the bitter gourd beetle, *Henosepilachna vigintioctopunctata* (Fab.).

2. Materials and Methods

2.1. Rearing of *Henosepilachna vigintioctopunctata*

Rearing of *H. vigintioctopunctata* was done to identify the various stages of the pest - egg, larva, pupa and adult and its duration, to carry out bioassay at a particular stage and to find out the efficacy of select medicinal plants. *H. vigintioctopunctata* is a major pest of bittergourd, *Momordica charantia*. Both the adults and larvae feed on the leaves, buds, flowers and fruits of bittergourd. Larvae of *H. vigintioctopunctata* collected from the infested bittergourd leaves at the Agricultural Farm Madras Christian College, Chennai, Tamil Nadu, were reared in the laboratory at room temperature 28 ± 2°C. The larvae were kept in a container (40cm x 25cm) and fed with fresh leaves of bittergourd. The emerged adults were separated out and kept as a male and female in ten jars. Fresh bittergourd twigs were kept inside the jars for oviposition. The jar was covered with muslin cloth. Eggs laid by the females were counted. Newly emerged larvae were fed with fresh leaves of bittergourd. Leaves were changed daily to avoid fungal infection to the larvae. Ten replications were maintained.

2.2. Collection of medicinal plants

Eight selected medicinal plants, *Acalypa indica*, *Andrographis paniculata*, *Cardiospermum halicacabum*, *Crotolaria retusa*, *Solanum surattense*, *Solanum trilobatum*, *Vitex negundo* and *Wedelia calendulacea* collected from various parts of Kanchipuram and Thiruvallur District, Tamil Nadu, India, were identified by Dr. C. Livingstone, Former Head, Department of Botany, Madras Christian College, Chennai, Tamil Nadu, India.

2.3. Solvent extraction of medicinal plants

The leaves of each plants were washed and shade dried. Dried leaves of each plants were powdered in an electric blender. The powder was extracted with hexane (Plate- 7-A) for a period of 48 hours at room temperature (28±2 °C) and filtered through a Whatman filter paper. The filtrate was evaporated to dryness under reduced pressure, using rotary vacuum evaporator (Plate- 7-B), weighed and stored at 4 °C for subsequent experiments. The remains of the plant material were extracted further with ethyl acetate and methanol, sequentially according to the polarity in a similar manner. All the solvents used were of analytical grade.

2.4. Isolation of active fractions

The most promising hexane crude extract of *A. paniculata* was subjected to column chromatography to isolate the fractions. 50gm of hexane crude extract was used in column chromatography over silica gel (500gm-Acme's silica gel, 100-200 mesh size) in hexane (Plate- X.A). The column was consecutively eluted with stepwise gradient of hexane 100%, followed by the combination of hexane: ethyl acetate at 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 100% ethyl acetate and finally the column was washed with 100% acetone. A total of 176 fractions were collected in 150ml conical flasks (Plate-X.B). Each fraction was spotted on a precoated silica gel (0.25mm thick TLC plate, E. Merck, Germany, 60 F254) for identification of different fractions. The fractions were spotted at 1cm from the edge of the sheet. The TLC plate was developed in a mixture of n-hexane and ethyl acetate to a distance of 8.8 cm and was dried at room temperature (28±2 ˚C). The TLC plate was visualized by UV light, Iodine vapor and Pancal D. The fractions with similar Rf values in TLC pattern were pooled together. Finally, eleven major fractions were obtained and fraction 4 formed as needle like crystals. The eluted fractions were stored at 4 ˚C for further bioassay.

2.5. Structure elucidation and identification

The active fraction was further analysed to ascertain, whether it contains single compound or mixture of compounds using TLC. Then it was subjected to spectroscopic analysis.

2.5.1. Ultraviolet –Visible Spectroscopy (UV)

An analytical method that measures the absorption of light in the 200 – 750 nm range of the electromagnetic spectrum.

2.5.2. Fourier Transform Infrared Spectroscopy (FT-IR)

Functional groups of purified compound were analysed using FTIR Spectroscopy (Perkin-Elmer spectrum RX-I FT-IR spectrometer in the range of 4000-450 nm). The purified compound was mixed either with chloroform or carbon tetrachloride for IR spectral analysis. Spectra of solid fractions were analysed by making transparent KBr pellets and liquid samples were recorded using Nujol mull (Fluka) on KBr windows.

2.5.3. GC-MS Analysis

By Mass Spectroscopy, the molecular mass of a compound and its elemental composition could be easily determined. Further this method involves very little amount of test sample, which gave molecular weights accurately. High resolution electron impact Mass Spectroscopy (EI-MS) was performed. The data system containing NIST 147 spectral library was used to verify the identity of individual components by mass spectral comparison.

2.5.4. Nuclear Magnetic Resonance Spectroscopy (NMR)
NMR is an analytical method that generates a spectrum that serves as the chemical signature of each molecule and aids in structure determination. The ^{1}H NMR spectra were recorded on Bruker instrument (AV 300 MHz) or JEOL instrument (GSX X 400 MHz) in CDCI3 (or) DMSO – d6 with tetra methyl saline (TMS) as the internal standard. Chemical shifts were recorded in δ scale.

2.6. Bioassay of active compounds
2.6.1. Antifeedant activity
Active compounds isolated were evaluated for their antifeedant activity at 250, 500, 750 and 1000 ppm concentrations against the test pest *H. vigintioctopunctata* as per the methodology described earlier (Arivoli and Tennyson, 2013) [15].

2.6.2. Larvicidal activity
Active compounds isolated were evaluated for their larvicidal activity at 250, 500, 750 and 1000 ppm concentrations against the test pest as per the methodology described earlier (Arivoli and Tennyson, 2013) [15].

2.6.3. Adulticidal activity
Active compounds isolated were evaluated for their adulticidal activity at 250, 500, 750 and 1000 ppm concentrations against the test pest *H. vigintioctopunctata* as per the methodology described earlier (Arivoli and Tennyson, 2013) [15].

2.7. Statistical analysis
Data collected from all the experiments were subjected to one way analysis of variance (ANOVA) in order to derive statistical significance of difference if any and the summary of ANOVA was provided. Mean values were separated into homogeneous subsets by post – hoc tests, when the F-value is significant. Statistical analysis was carried out using SPSS 17.0.

3. Results and Discussion
3.1. Isolation, identification and structure elucidation of compounds from active fraction
Eleven fraction were obtained from GC-MS analysis, fourth fraction exhibited maximum antifeedant and larvicidal activity. So it was further subjected to GC-MS analysis. The GC-MS analysis result showed in Fig. 1 revealed the presence of 6 compounds 5.92% ergost-5-en-3-ol, (3.beta.)-, 30.02% stigmasterol, 41.55% beta-sitosterol, 17.49%. Gamma-sitosterol, 2.95% fucosterol and 2.08 stigmasta-5, 24 (28)-dien-3-ol, (3.beta.) in fraction 4. To isolate the major compounds, fourth fraction was rechromatographed using hexane; ethyl acetate solvent system, and 32 sub fractions (32 ×150 ml) were obtained. In the light of different spectral analysis, AP-1 was identified as Stigmasterol ($C_{29}H_{48}O$) and AP-2 as β-sitosterol ($C_{29}H_{50}O$). Structure of stigmasterol was given in Fig. 2a and β-sitosterol was given in Fig. 2b.

3.2. Stigmasterol
Melting point (mp): 176˚C, Molecular formula (mf): $C_{29}H_{48}O$, Mass m/z: 412

Spectral analysis
UV λmax MeOH nm: 244 ; IR νmax KBr cm-1: 3410, 2955, 2956, 2903, 1634, 1451, 1383, 1443, 1250, 1193, 1095, 1023, 971, 961, 805, 800, 682 (Fig. 3a).

MASS spectrum
The MASS spectral fragmentation pattern of stigmasterol was given in (Fig. 3b)

^{1}H NMR
In ^{1}H NMR spectrum of stigmasterol (Fig. 3c) H-3 proton appeared as a triplet of a double doublet (tdd) at δ 3.25 (J = 4.5 and 1.1 MHZ) and H-6 olefenic proton showed a multiplet at δ 5.14. Two olefenic protons appeared downfield at δ 4.62 (m) and δ 4.61 (m) which were identical with the chemical shift of H-22 and H-23, respectively of stigmasterol. Six methyl protons also appeared at δ 1.07, δ 1.26, δ 0.91, δ 1.01, δ 1.00 and δ 0.97.

^{13}C NMR
^{13}C NMR (CDCl3) δ: 141.62 (C-5), 137.65 (C-23), 129.29 (C-6), 120.17 (C-22), 77.4 (C-3), 70.25 (C-17), 56.53 (C-14), 55.87 (C-24), 50.62 (C-4), 50.8 (C-9), 42.4 (C-13), 42.03 (C-10), 40.33 (C-2), 39.38 (C-20), 37.17 (C- 1), 36.32 (C-12), 31.79 (C-15), 31.57 (C-25), 31.26 (C-8), 28.92 (C-28), 24.71 (C-15), 23.94 (C-16), 21.04 (C-26), 20.77 (C-27), 20.60 (C-11), 19.10 (C-18), 18.90 (C-24), 11.92 (C-21), 11.80 (C-29) (Fig. 3d).

3.3. β-Sitosterol
Melting point (mp): 133˚C, Molecular formula (mf): $C_{29}H_{50}O$, Mass m/z: 414

Spectral analysis
UV λmax MeOH nm: 250; IR νmax KBr cm-1: 3430, 2937, 1642, 1464, 1381, 1054, 958, 801. (Fig. 4a).

MASS spectrum
The MASS spectral fragmentation pattern of β-sitosterol was given in Fig. 4b.
^{1}H NMR
In 1H NMR spectrum of β-sitosterol (Fig. 4c) H-3 proton appeared at δ 3.2 as a triplet of a double doublet with a J value of 4.5 and 1.1 MHZ and H-6 olefinic proton showed a multiplet at δ 5.31. Moreover, six methyl protons appeared at δ 1.16, δ 1.25, δ 0.91, δ 1.01, δ 0.91 and δ 1.26 (3H each, s, CH_3).

^{13}C NMR
^{13}C NMR (CDCl3)δ: 140.74 (C-5), 121.70 (C-6), 77.42 (C-3), 77.00 (C-17), 76.56 (C-14), 71.79 (C-4), 56.75 (C-9), 56.05 (C-24), 50.12 (C-13), 45.8 (C-10), 42.2 (C-2), 39.7 (C-22), 37.24 (C-1), 36.49 (C-12), 36.1 (C-7), 33.93 (25), 31.89 (C-20), 31.65 (C-23), 29.14 (C-8), 28.23 (C-28), 26.07 (C-15), 24.29 (C-16), 23.05 (C-11), 21.07 (C-18), 19.81 (C-19), 19.38 (C-26), 19.02 (C-27), 18.77 (C-21), 11.97 (C-29) (Fig 4d).

3.4. Bioassays of active phytocompounds
3.4.1. Antifeedant activity
In the present work, isolated compounds stigmasterol and β-sitosterol were evaluated for their antifeedant activity at 250, 500, 750 and 1000 ppm concentrations against *H. vigintioctopunctata*. Stigmasterol at 1000 ppm exhibited a higher phagodeterrency of 67.83% than β-sitosterol (55.87%) (Table 1). Positive control azadirachtin showed 93.27% of feeding deterrency and no effect was observed in negative

control. Stigmasterol manifested higher antifeedant activity at all concentrations than β-sitosterol. The mean variation is statistically significant by Tukey's test with $P < 0.05$ level and ANOVA. Both compounds exhibited a dose-dependent effect of antifeedancy. These findings correlate with the findings of Huang *et al*. (2008) [16] when they have treated *Plutella xylostella* with stigmasterol at 2000 µg/ml and have observed antifeedant index of 44.26%. Champagne and Bernays (1991) [17] have reported *Schistocerca americana* was capable to regulate feeding in response to unsuitable stigmasterol. *Schistocerca americana* presented with diet containing stigmasterol exhibited strong deterrent responses**.** Mamta *et al*. (1997) [18] have reported significant antifeedancy with stigmasterol against *Spodoptera litura* and *Spilosoma oblique*. They have treated *Schistocerca americana* sixth stadium nymphs with sitosterol and observed the feedeing deterrency of 44.8% (Behmer and Elias, 1999a) [19].

3.4.2. Larvicidal activity

The larvicidal effect of compounds stigmasterol and β-sitosterol were evaluated at 250, 500, 750 and 1000 ppm concentrations against *H. vigintioctopunctata*. Stigmasterol exhibited maximum larval mortality of 76.0% at 1000 ppm and least effect of 24.0% at 250 ppm (Table 2). The LC_{50} and LC_{90} values were 528.288 and 1855.351ppm. The Chi-square values and Tukey's test were significant at $P < 0.05$ level. Maximum and minimum effects of β-sitosterol were 50.0 and 18.0 at 1000 and 250 ppm, respectively. The LC_{50} and LC_{90} values were 1257.29 and 9975.896ppm. Both stigmasterol and β-sitosterol exhibited larval mortality in a dose-dependent manner. Statistically significant difference was noticed with in stigmasterol, β-sitosterol, positive control and negative control by ANOVA and Tukey's post hoc test $P < 0.05$. The present findings coincide with the findings of Behmer and Grebenok (1998) [20] who have reported that survival of *Plutella xylostella* larvae on stigmasterol diet was only 55.0%. This is in accordance with the findings of Behmer and Elias (1999b) [21] who have treated *S. americana* with 0.025% sitosterol and observed survival was below 50.0%. *Diatraea grandiosella* had lower survival on stigmasterol (57.0%) relative to sitosterol (80.0%) (Chippendale and Reddy, 1972) [22].

3.4.3. Adulticidal activity

Stigmasterol and β-sitosterol were tested for their adulticidal activity at 250, 500, 750 and 1000 ppm concentrations against *H. vigintioctopunctata*. Maximum adult mortality of 84.0% at 1000ppm and least effect of 48.0 % at 250 ppm (Table 3) with LC_{50} and LC_{90} values of 285.111 and 2013.59 ppm were observed in stigmasterol. The ANOVA, Chi-square values and Tukey's test were significant at $P < 0.05$ level and a lower mortality of 22.00 % at 250 ppm with LC_{50} and LC_{90} values of 885.081 and 8445.071 ppm. Negative control did not exhibit adulticidal activity. Significant adulticidal activity was exhibited by stigmasterol isolated from the extract of *Ajuga nipponensis* against *Plutella xylostella* (Huang *et al*., 2008) [16]. β-sitosterol exhibited 50.0% mortality at 1000 ppm concentration in the present study. The finding correlates with the findings of Behmer and Elias (1999b) [21] who have observed that sitosterol at 2.0mg/g produced significant adult mortality against *Schistocerca americana*.

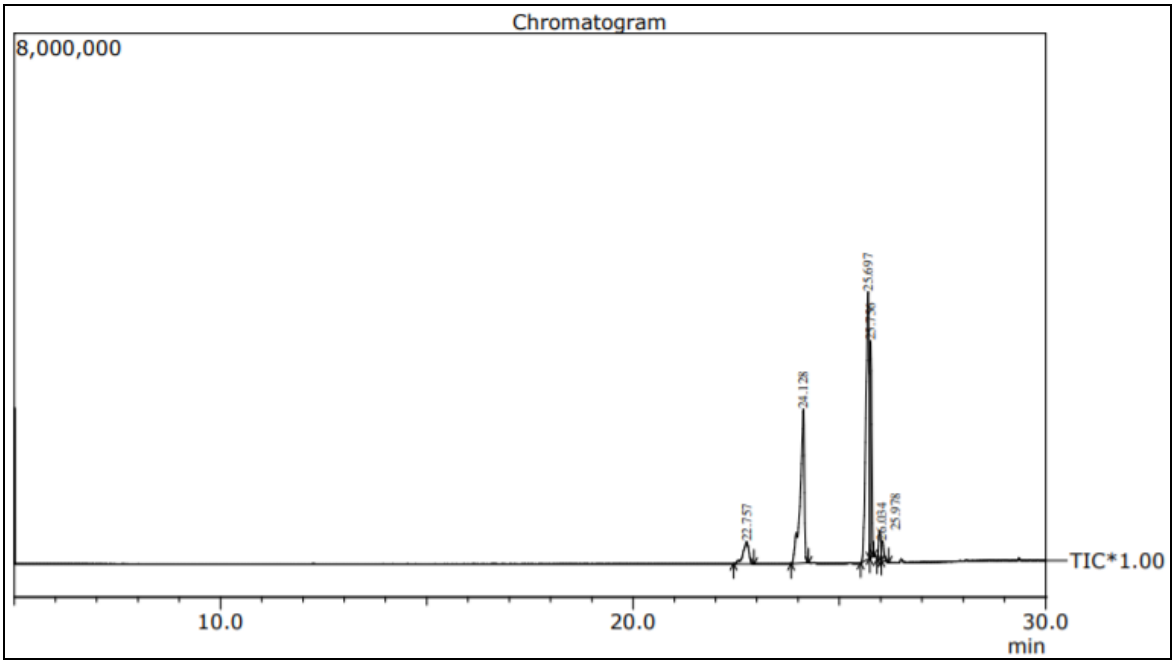

Fig 1: GC-MS analysis of active fraction isolated from crude extract of *A. paniculate*

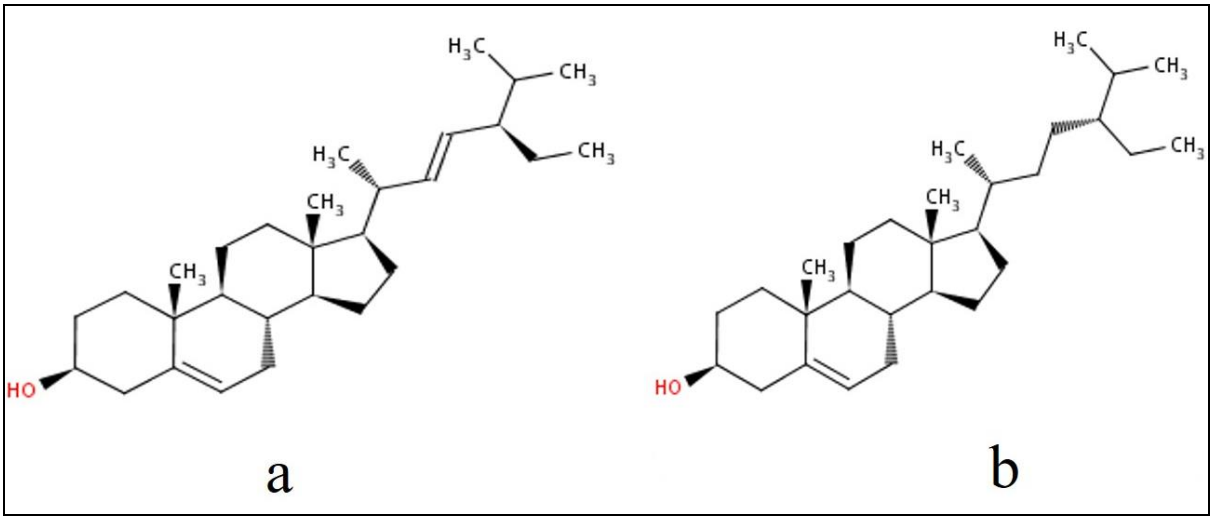

Fig 2: Chemical structure a) Stigmasterol b) β-sitosterol

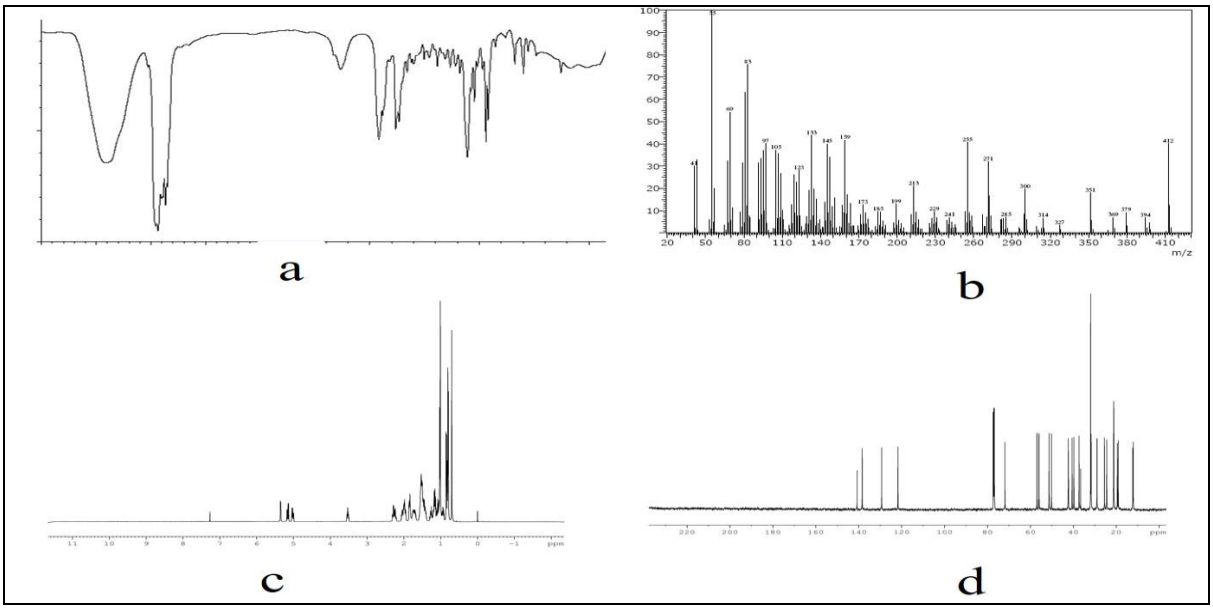

Fig 3:a) FT-IR spectrum **b)** Mass spectrum **c)** ^{1}H NMR **d)** ^{13}C NMR of stigmasterol

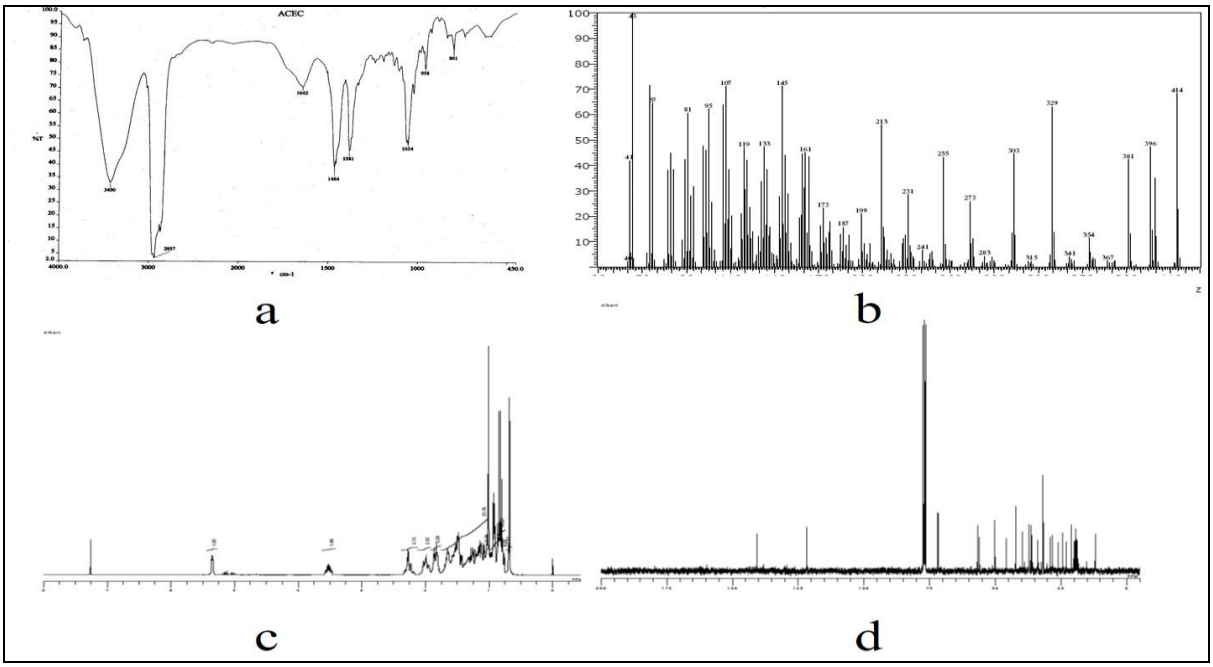

Fig 4: a) FT-IR spectrum **b)** Mass spectrum **c)** 1H NMR **d)** ^{13}C NMR of β-sitosterol

Table 1: Antifeedant activity of compounds isolated from *A. paniculata* against *H. vigintioctopunctata*

Compounds	Concentration (ppm)			
	250	500	750	1000
Stigmasterol	39.67 [a] ± 1.37	45.3 [a] ± 5.55	59.70 [b] ± 5.47	67.83 [c] ± 3.40[c]
β-sitosterol	26.20 [a] ± 3.13	35.45 [b] ± 2.97	47.90 [c] ± 5.14	55.87 [d] ± 5.02
Azadirachtin	59.39 [a] ± 5.49	67.42 [a] ± 3.64	75.33 [b] ± 7.58	93.27 [c] ± 3.06
Control	0.0 [a]	0.0 [a]	0.0 [a]	0.0 [a]

Values are (Mean ± SD) of five replications. Means followed by the same letter do not differ significantly (Tukey's test, P ≤ 0.05).

Table 2: Larvicidal activity of compounds isolated from *A. paniculata* against *H. vigintioctopunctata*

Compounds	Concentration (ppm)			
	250	500	750	1000
Stigmasterol	24.0 [a] ± 8.94	44.0 [b] ± 5.47	64.0 [c] ± 8.94	76.0 [c] ± 5.47
β-sitosterol	18.0 [a] ± 4.47	26.0 [ab] ± 5.47	32.0 [b] ± 8.36	50.0 [c] ± 7.07
Azadirachtin	36.0 [a] ± 5.47	54.0 [b] ± 5.47	70.0 [c] ± 7.07	82.0[d] ± 4.47
Control	0.0[a]	0.0 [a]	0.0 [a]	0.0 [a]

Values are (Mean ± SD) of five replications. Means followed by the same letter do not differ significantly (Tukey's test, P ≤ 0.05).

Table 3: Adulticidal activity of compounds isolated from *A. paniculata* against *H. vigintioctopunctata*

Compounds	Concentration (ppm)			
	250	500	750	1000
Stigmasterol	48.0 [a] ± 4.47	64.0 [b] ± 5.48	68.0 [b] ± 4.47	84.0 [c] ± 5.47
β-sitosterol	22.0 [a] ± 8.37	40.0 [b] ± 7.07	48.0 [b] ± 8.36	50.0 [b] ± 7.07
Azadirachtin	44.0 [a] ± 5.46	64.0 [b] ± 8.94	76.0 [b] ± 5.48	92.0 [c] ± 8.37
Control	0.0[a]	0.0 [a]	0.0 [a]	0.0 [a]

Values are (Mean ± SD) of five replications. Means followed by the same letter do not differ significantly (Tukey's test, P ≤ 0.05).

4. Conclusions

In the present study, *A. paniculata* exhibited maximum phagodeterrency of 56.54% and maximum larval mortality of 62% with low polar hexane extract. Antifeedant and Larvicidal activity was observed in the *A. paniculata* is 56.54 and 62%. Fraction 4 exhibited maximal larval mortality of 70.0% at 1000 ppm concentration with LC_{50} and LC_{90} values were 601.65 and 4305.106 ppm. No larvicidal effect was observed with the fractions ten and eleven at all concentrations. Stigmasterol at 1000 ppm exhibited maximum phagodeterrency of 67.83%. Larvicidal activity was more significant in stigmasterol than β-sitosterol. Maximum 84.00% adulticidal activity was observed with stigmasterol at 1000 ppm with LC_{50} and LC_{90} values were 285.111 and 2013.591 ppm. It has been concluded that possibility of employing *A. paniculata* derived active phytocompounds to manage the bittergourd pest *H. vigintioctopunctata* may be worthy for further field evaluation.

5. Acknowledgements

Authors thank the authorities of Sri Vasavi College, Erode for providing the essential infrastructure facilities needed for the study.

6. References

1. Pimentel D, Techniques for Reducing Pesticide Use. Economic and Environmental Benefits (J. Wiley & Sons, New York, 1997.
2. Jayaraj S. Host plants resistance derived through conventional and biotechnological means for insect Pest management In: Biotechnology and Insect Pest management (Eds.S. Ignacimuthu and S. Jayaraj) Elite publishing House, New Delhi 2007, 1-10.
3. Erturk O. Antifeedant and toxicity effects of some plant extracts on *Thaumetopae solitaria* Frey. (Lepidoptera: Thaumetopoeidae). Turkish Journal of Biology. 2006;30:51-57.
4. Bami HL. Pesticide use in India – Ten questions. Chemistry Weekly 1997;4:7-10.
5. Gupta PK. Pesticide exposure - Indian Scene Toxicology. 2004;198(1-3):83-90.
6. Kannaiyan S. Insect pest management strategies: Current trends and future prospects. In: Strategies in Integrated Pest Management. (Eds. S. Ignacimuthu, and Alok Sen) Phoenix Publishing House. New Delhi. 2002, 1-13.
7. Leatemia JA, Isman MB. Efficacy of crude extracts of Annona squamosa against diamond back moth, *Plutella xylostella* L. in the greenhouse. International Journal of Pest Management. 2004;50:129-133.
8. Nosalovaa G, Majeeb SK, Ghoshb K, Rajab W, Chatterjeeb UR, Jureceka L, Ray B. Antitussive arabinogalactan of *Andrographis paniculata* demonstrates synergistic effect with andrographolide. International Journal of Biological Macromolecules. 2014;69:151-157.
9. Govindarajan M. Evaluation of *Andrographis paniculata* burm.f. (Family: acanthaceae) extracts against *Culex quinquefasciatus* (Say.) and *Aedes aegypti* (Linn.) (Diptera: culicidae). Asian Pacific Journal of Tropical Medicine 2011;4(3):176-181.
10. Palada MC, Chang LC. Suggested cultural practices for bittergourd. Internat. Co-operators guide. AVRDC pub 2003.
11. Shahid A. Andrographis paniculata: A review of pharmacological activities and clinical effects. Alternative Medicine Review 2011;16(1): 66-77.
12. Paul A, Raj VS, Vibhuti A, Pandey RP. Larvicidal efficacy of *Andrographis paniculata* and *Tinospora cordifolia* against *Aedes aegypti*: A dengue vector. Pharmacognosy Research 2020;12(4): 352.
13. Baliyarsingh B, Mishra A, Rath S. Evaluation of insecticidal and repellency activity of leaf extracts of *Andrographis paniculata* against *Tribolium castaneum* (Red flour beetle). International Journal of Tropical

Insect Science 2021;41(1):765-773.
14. Vattikonda SR. Effect of Andrographolide on feeding behaviour of *Papilio demoleus* L., (Lepidoptera: Papilionidae) larvae. Asian Journal of Bio Science 2015;10(1):65-70.
15. Arivoli S, Tennyson S. Antifeedant activity, developmental indices and morphogenetic variations of plant extracts against *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae). Journal of Entomology and Zoology Studies 2013;1(4):87-96.
16. Huang Z, Zhou FC, Xu D, Afzal M, Bashir MA, Ali S, *et al*. Antifeedant activities of secondary metabolites from *Ajuga nipponensis* agaist *Plutella xylostella*. Pakistan Journal of Botany 2008;40(5):1983-1992.
17. Champagne DE, Bernays EA. Phytosterol unsuitability as a factor mediating food aversion learning in the grasshopper *Schistocerca Americana*. Physiological Entomology 1991;16:391-400.
18. Mamta T, Shukla YN, Tripathi AK, Singh SC. Insect antifeedant principles from Vernonia cinera. DOI 10.1002/(SICI)1099 – 1573 (199805) 12:3<195::AID – PTR223>3.0.CO;2-#.1997.
19. Behmer ST, Elias DO. Phytosterol structure and its impact on feeding behaviour in the generalist grasshopper *Schistocerca Americana*. Physiological Entomology. 1999a;24:18-27.
20. Behmer ST, Grebenok RJ. Impact of dietary sterols on life history traits of a caterpillar. V. Physiological Entomology 23:165-175.
21. Behmer ST, Elias DO. The nutritional significance of sterol metabolic constraints in the generalist grasshopper *Schistocerca americana*. Journal of Insect Physiology. 1999b;45:339-348.
22. Chippendale GM, Reddy GPV. Polyunsaturated fatty acid and sterol requirements of the Southwestern cornborer, *Diatraea grandiosella*. Journal of Insect Physiology 1972;18:305-316.