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Research Article

ACACIA AURICULIFORMIS: A GAMUT OF BIOACTIVE CONSTITUENTS AGAINST BACTROCERA CUCURBITAE

Amandeep Kaur[1*], Satwinder Kaur Sohal[2], Saroj Arora[3] and Harpreet Kaur[2]

[1] Department of Zoology, Dolphin (PG) College of Life Sciences, Chunni Kalan, Fatehgarh Sahib, Punjab-140406, India
[2] Department of Zoology, Guru Nanak Dev University, Amritsar, Punjab-140405, India
[3] Department of Botanical & Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab-140005, India

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ABSTRACT

The traditional pest control strategies led the world to the era of pesticide contaminated food, air and water. The main focus of the scientific community is to develop ecofriendly, biodegradable and safer to mankind chemicals for pest control. This search results in exploration of plants for their vast phytochemical resources. The present study forms a hypothesis of testing active ingredients of an economic medicinal plant, *Acacia auriculiformis*, against the melon fruit fly, *Bactrocera cucurbitae.* The results concluded the promising effect of fractions of ethyl acetate extract against the insect pest as they pronouncedly reduce the emergence, enhanced the mortality and decrease the pupal weight of pest with treatments. The chemical characterization of the active ingredients with different spectroscopic techniques (FTIR, NMR, LC-MS) indicate presence of flavonoids as the major portion of the fractions.

Keywords: *Acacia auriculiformis, Bactrocera cucurbitae*, Pesticides, FTIR, NMR, LC-MS.

INTRODUCTION

Synthetic chemical pesticides used for pest control in agriculture have resulted in enormous problems raising concerns about their effect on environment and human health. It necessitates imposing a limit on the use of these harmful chemicals. But the efficient control of these pests is also of the utmost importance to ensure the adequate food supply for the ever increasing population of the world. The scientists and industrialist focus their concerns towards the research of alternatives of conventional chemicals used for pest control. These efforts direct the researchers towards phytochemical screening for developing biodegradable, ecofriendly and safer compounds for pest control. Rich flora has evolved a wide gamut of compounds, which are not directly involved in normal metabolism of the plant but synthesized as byproducts of the normal growth and metabolic pathways. These secondary phytochemicals usually considered as the defensive compounds of the plant. A number of these compounds from a variety of plants have been explored for their bioactivity against many pest species. Among the variety of secondary metabolites, Phenolic compounds share the major portion. Phenolics are the main defensive compounds of the plants army against herbivore attack. The reports available have demonstrated varied responses of phenolics to herbivores, ranging from negative in about half of the cases to positive or neutral in the other half. The negative consequences of phenolics to herbivores include potential anti digestive effects, inhibition of crucial digestive enzymes, production of midgut lesions by oxygen free radical and feeding deterrence (Bilal and Hassan, 2012; Ghosh *et. al.* 2012; Khater, 2012; Mann and Kaufman, 2012). On the other hand, insects, being coevolved with plants, counter various measures to cope with toxic phytochemicals.

Acacia auriculiformis A. Cunn. (Black wattle or Australian kikkar) is an important medicinal plant and widely distributed member of family Fabaceae. Its rich secondary metabolite metabolites include phenolics, tannins and terpenoids mainly. Its anti-helminthes, anti-fungal and anti-microbial effects have been well documented by several researchers (Kazhila and Marius, 2010; Mandel *et. al.* 2005; Sathya and Siddhuraju, 2012). The insecticidal effect of

*Corresponding author e-mail: amandeepzoology@gmail.com, Mobile: +91 9915380006

crude extracts of the plant is also well established (Kaur *et. al*. 2009; 2010). But the purified fractions are least explored for their bioactivity against insects. Melon fruit fly, *Bactrocera cucurbitae* (Coquillett) is an important economic pest of vegetable crops. It can cause a loss ranged from 30 to 100% to the crop yield to approximately 70 host plants throughout the year (Dhillon *et. al*. 2005).

The above mentioned facts necessitates the investigations of insecticidal potential of purified fractions obtained form *A. auriculiformis* against the selected pest, *B. cucurbitae* along with the analysis of chemical constitutes of these fractions to establish a base for industrial applications and formulations of ready to use type of chemicals for farmers.

MATERIALS AND METHODS

Procurement and extraction of plant material

A. auriculiformis was identified by comparing it with the specimen available in the herbarium (Voucher number 6422) of the Guru Nanak Dev University, Amritsar. The bark of *A. auriculiformis* was procured and washed with tap water (thrice) and dried in oven at 30ºC for overnight in order to ensure complete drying and then ground to a fine powder with grinder. The extracts of *A. auriculiformis* were prepared by maceration extraction method using protocol in which the bark was extracted with solvents in order of increasing polarity *i.e.* ethyl acetate, acetone, methanol and water resulted in six different bark extracts as *A. auriculiformis* Hexane extract (AAH), *A. auriculiformis* Chloroform extract (AAC), *A. auriculiformis* Ethyl acetate extract (AAE), *A. auriculiformis* Acetone extract (AAA), *A. auriculiformis* Methanol extract (AAM) and *A. auriculiformis* Water extract (AAW).

Purification of AAE

AAE of *A. auriculiformis* having the lowest LC_{50} value was subjected to column chromatography and thin layer chromatography to isolate the different fractions.

Column Chromatography

Aluminium oxide neutral (350g) was packed in a column (size 75cm x 3.5cm) with pure hexane. The pure hexane was run four to five times through the aluminium oxide neutral in the column to ensure efficient packing. 25g of AAE-W fraction was dissolved in minimum volume of methanol and to this 25g of Celite '545' was added. The slurry was completely dried and loaded onto the aluminium oxide column. The non-absorbent cotton was put on the slurry, to avoid any disturbance on adding solvent into the column. On eluting the column with pure hexane, 20 fractions of 50ml each were collected and thin layer chromatography (TLC) was performed in pure hexane. All the fractions were pooled because there was no spot on the TLC plate with pure hexane. The polarity of the eluting solvent was enhanced by adding 10% ethyl acetate in hexane and 21-55 fractions of 50ml each were collected. Different fractions (50ml each) were collected with eluting solvents (hexane, chloroform, ethyl acetate, methanol, acetone and water) having different polarities. The fractions were pooled, dried and then subjected to column chromatography again. The whole column was eluted with mixture of different solvent systems of varying polarities.

Thin Layer Chromatography (TLC)

Thin layer chromatography was done on pre-coated Kieselgel 60 F_{254} –plates (0.2mm thick; Merck, India). The different extracts/fractions of the plant were dissolved in methanol and were spotted on the plate with fine capillary tube at the height of 0.8-1.0cm from the base. In the present study, different solvent mixtures viz; chloroform, ethyl acetate, methanol, acetone and their mixtures were used for developing the TLC plates to get better results. After putting the plates in solvent system, the appropriate distance moved by the solvent was measured to find retention factor (nearly 2/3 of plate). The plates were air dried and developed with iodine in iodine chamber, under UV light nm_{254} and nm_{360} and spraying the TLC plates with 10% sulphuric acid. The Retention Factor (Rf) values of all compounds isolated were calculated. The fractions with similar Rf values were pooled and isolates designated as A1, A2 and A4 were obtained. All the isolated fractions were stored in solid form for further experimentation.

$$Rf = \frac{\text{Distance moved by the spot}}{\text{Distance moved by the solvent front}}$$

Melting point determination

A thin glass capillary tube containing a compact column of the sample was introduced into a heated stand (a beaker half filled with paraffin wax) in close proximity to a high accuracy thermometer. The temperature in the heating stand was ramped at a fixed rate until the sample in the tube transitions into the liquid state. The temperature at this stage was noted and considered as melting point of the sample.

Chemical characterization of fraction isolates

The structures of fraction isolates (A1, A2 and A4) were determined by spectroscopic techniques *i.e.* Nuclear Magnetic Resonance (^{1}H-NMR), Fourier Transformation Infrared (FTIR) and Mass spectroscopy. All these characterization techniques were performed at central instrumentation department of Punjab University, Chandigarh. The nuclear magnetic resonance spectroscopy was recorded on Jeol 400MHz spectrophotometer. For structural analysis of isolated compounds, FTIR absorption spectra were recorded at room temperature in the 400-4000cm^{-1} range using a spectrophotometer Shimadzu FTIR-8700.

Laboratory Rearing of Melon Fruit Fly

The wild culture of melon fruit fly was procured from the infested bitter gourds, *M. charantia* collected from the kitchen gardens of university campus and vegetable market of Amritsar city. The freshly emerged flies from the infested bitter gourds were identified on the basis of its taxonomic characters given by Kapoor (1993). The flies were reared in the laboratory according to the requirement of the experiments on natural and artificial diet in insect culture room/B.O.D under controlled temperature (25±2ºC), relative humidity (70-80%) and photoperiod (10L:14D).

Insect Rearing on Natural Food

The methodology of Gupta and Verma (1978) was used for rearing the larvae of melon fruit flies on natural food under controlled laboratory conditions.

Insect Rearing on Artificial Diet

The experiments on fruit fly larvae were done with artificial diet according to the standardized methodology given by Srivastava (1975).

Experiments with Larvae

The experiments were performed with second (64-72h old) instar larvae. About 100 gravid females were released in wire mesh cages containing fresh pumpkin pieces for an interval of 8h. The charged pumpkin pieces were dissected in saline water for harvesting the larvae after 64h of removal of the fruit. The harvested larvae were transferred to culture vials (D 25mm x L 100mm) containing medium treated with various concentrations of different plant extracts and fraction isolates. The experimental vials were kept in culture room/B.O.D. The larvicidal activity of the extracts was evaluated by recording the number of pupae formed and number of flies emerged. LC_{50} concentration was calculated by the probit analysis method of Finney (1971) using SPSS computer based software. There were six replications with 20 larvae in each replication for each concentration as well as control and the experiments were repeated twice.

Statistical analysis

The data was computed and statistically analyzed by using SPSS software and the statistical tests according to the requirements of the experiments.

RESULTS

The bioassay experiments with the crude bark extracts of plant resulted in maximum larval mortality with AAE treatment as it gives lowest LC_{50} value (Table 1).

Effect of fraction A1 on *B. cucurbitae*

Development: Fraction A1 prolonged the larval period significantly ($F_{(df=4,25)}$=16.009) when second instar larvae (64-72h old) of *B. cucurbitae* were given treatment. Maximum prolongation in the larval period was observed at 64ppm where it got delayed by 3.69 days. On the other hand, the pupal period ($F_{(df=4,25)}$=4.909) and total development period ($F_{(df=4,25)}$=25.788) shortened considerably with treatment but the reduction was not concentration dependent (Table 2).

Pupal weight: The weight of the pupae formed from 64-72h old treated with fraction A1 decreased significantly ($F_{(df=4,25)}$=6.142) as compared to control. The pupal weight which

was 18mg in control decreased to 14.97mg at 256ppm concentration (Table 2).

Larval and pupal mortality: The deleterious effect of fraction A1 was manifested in the increased larval and pupal mortality observed at all the concentrations of the fraction. The larval mortality which was 13.33% in the control showed a 7.3 fold increase at the higher concentrations. The pupal mortality too increased 3.7 times that of control in the treated larvae (Table 2).

Effect of fraction A2 on *B. cucurbitae*:

Development: The fraction A2 significantly shortened the larval period ($F_{(df=4,25)}$=4.897), pupal period ($F_{(df=4,25)}$=17.039) and total development period ($F_{(df=4,25)}$=54.270) of the second instar larvae (64-72h old) fed on treated diet. The decline observed in the larval, pupal and total development period showed no correlation with the concentration of the fraction (Table 3).

Pupal weight: Pupal weight was reduced significantly ($F_{(df=4,25)}$=5.78) when the larvae (64-72h old) were treated with A2 fraction containing A2 as the predominant phenolic compound.

At 625ppm concentration, the decrease in pupal weight was found to be 82.05% of the control (Table 3).

Larval and pupal mortality: Significant effects of A2 fraction (A2) were observed on percent larval mortality ($F_{(df=4,25)}$=587.71) and percent pupal mortality ($F_{(df=4,25)}$=430.83) when the second instar larvae (64-72h old) were given treatment. Both larval and pupal mortality increased considerably after treatment at all concentrations but showed no correlation with concentration. While the larval mortality showed a 5.2 fold increase, the pupal mortality showed a 3.09 fold increase when observations were made at the highest concentration of 256ppm (Table 3).

Effect of fraction A4 on *B. cucurbitae*:

Development: Fraction A4 significantly reduced the pupal period ($F_{(df=4,25)}$=11.08) and the total development period ($F_{(df=4,25)}$=25.516) of the second instar larvae (64-72h old) but had no significant effect on the larval period. The pupal period was shortened by 4.06 days at 256ppm, while the total development period decreased by 4.29 days at the same concentration (Table 4).

Pupal weight: The weight of the pupae formed decreased when the second instar larvae (64-72h old) were treated with fraction A4. Maximum decrease was observed at 256ppm where the pupal weight declined to 85.96% of the control (Table 4).

Larval and pupal mortality: The larval and pupal mortality was found to be significantly greater in the second instar larvae (64-72h old) treated with fraction A4 as compared to control. The mortality was high even at the lower concentrations indicating the toxic effect of the fraction (Table 4).

Chemical characterization of fraction isolates

Fraction A1

A yellow brownish solid (267mg) having a melting point of 285-287^{0}C was assigned the presence of A1 (5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one) as the predominant compound with chemical formula $C_{15}H_{10}O_5$. Mass ion peak of this fraction was obtained at ESI-MS m/z 271 (M^+H, 100%) (Fig. 1) and Rf value of 0.60. The FTIR spectrum (Fig. 2) of A1 showed strong absorption bands at 1384.3cm^{-1} and 1443.6cm^{-1} represented ether linkage, -CH- and bending of CH group, respectively. Other strong absorption bands at 1507.9cm^{-1} and 1714.6cm^{-1} represented C=C aromatic ring and carbonyl groups, respectively. At 1618.1cm^{-1}, a very strong band of aromatic ring representing C=C alkenes and a broad band at 3396cm^{-1} attributed to OH group was obtained in the spectrum. The ^{1}H NMR spectrum (Fig. 3) exhibited singlet at δ6.12 (H-6), doublet at δ6.4 (J=0.8, H-8), singlet at δ6.7 (H-3), doublet at δ6.9 and δ7.8 with J= 8.4 and representing H-3', 5' and H-2', 6', respectively.

Fraction A2

Fraction A2 was isolated as a brown amorphous powder. The major compound in this fraction was A2 (3, 4, 5-trihydroxybenzoic acid) with molecular formula $C_7H_6O_5$. The chemical structure was given on the basis of ESI-MS m/z: 170.178 (M^+H, 100%) (Fig.4). The uncorrected melting point of A2 was 250-252^{0}C and Rf value was 0.45. The FTIR spectrum exhibited nine different absorption bands (Fig. 5). Two strong

bands at 841.7cm^{-1} and 1031.9cm^{-1} represented C-H and –C-O. A weak band at 1339.7cm^{-1} represented C-OH. A very strong band of 1455.1cm^{-1} corresponded to bending vibrations of CH. A phenyl aromatic ring at 1507.9cm^{-1}, C-H or C=C at 1616.3cm^{-1}, symmetrical stretching of -C-H at 2852.9cm^{-1} and asymmetrical stretching of –C-H at 2923.0cm^{-1} was observed in the spectrum. A very weak absorption band of 3258.4cm^{-1} was assigned to OH group. The ^{1}H NMR spectrum (Fig.6) exhibited multiplet of δ3.83-4.75 (OH, H-3), δ7.0 (singlet for aromatic ring, H-2,6) and δ9.21 (singlet for COOH, H-3,5).

Fraction A4

A brownish yellow powder (37mg) with Rf value of 0.44, uncorrected melting point 237-240^{0}C and ESI-MS m/z 290.3 (M^{+}H, 100%) with chemical formula $C_{15}H_{14}O_6$ represented the major peak of A4 ((2R,3R)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol) in fraction A4 as the major compound in fraction of A4 (Fig.7). The structure of this compound was further confirmed with FTIR and NMR spectral analysis. FTIR spectrum of A4 (Fig.8) exhibited eight absorption bands. A very weak band at 3396.2cm^{-1} represented O-H group. Four weak absorption bands represented symmetrical deformation of CH_2 and COH group (1387.1cm^{-1}), bending vibrations of CH (1455.9cm^{-1}), phenyl ring (1508.9cm^{-1}) and symmetrical –C-H (2852.4cm^{-1}). Three strong bands at 1112.7cm^{-1}, 1617.3cm^{-1} and 2922.5cm^{-1} represented COH, C-H or C=C and asymmetrical –C-H (CH_2) stretching, respectively. ^{1}H NMR spectrum (Fig. 9) of A4 showed aromatic signals at δ5.83, δ6.10, δ6.85 and δ7.15. One doublet with coupling constant of 8Hz represented CH group at δ4.33.

Table 1. Extracts of *A. auriculiformis* and their LC_{50} value.

Extracts	LC_{50} (ppm)	Regression Line Equation (y= mx+c)	R^2
AAM	290.82	3.8647x + 32.494	0.9896
AAE	187.13	9.3333x + 10.224	0.9546
AAA	273.60	6.0486x + 22.789	0.9769
AAW	306.46	8.4086x + 6.1339	0.9325

X = explanatory variable, ***Y*** =dependent variable, slope of line = ***m***, ***c*** =intercept

Table 2. Effect of fraction A1 on development of second instar larvae (64-72h old) of *B. cucurbitae.*

Concentrations	Larval Period (Days)	Pupal Period (Days)	Total Developmental Period (Days)	Pupal Weight (mg)	Percent Larval mortality	Percent Pupal mortality
Control	9.50±0.28 ^{b}	11.15±0.349 ^{a}	20.65±0.168 ^{a}	18.00±1.41^{a}	13.33± 2.31^{b}	26.67±3.65^{b}
4 ppm	6.40±0.286 ^{c}	7.30±0.523 ^{ab}	13.70±0.748 ^{c}	15.00±0.35^{b}	94.84± 0.77^{a}	98.50±.37^{a}
16 ppm	10.44±0.52 3 ^{b}	6.06±0.423 ^{b}	16.50±0.408 ^{b}	15.00±0.00^{b}	97.34± 0.54^{a}	98.84±0.52^{a}
64 ppm	13.19±0.80 1 ^{a}	6.31±1.935 ^{b}	17.83±0.182 ^{b}	14.77±0.18^{b}	97.34± 0.46^{a}	98.84±0.52^{a}
256 ppm	10.72±0.899 ^{ab}	6.18±0.865 ^{b}	16.90±0.523 ^{b}	14.97±0.39^{b}	97.17±0.34^{a}	98.67±0.36^{a}
F- Value	16.009**	4.909**	25.788**	6.142**	1273.94**	440.06**

**Significant at 1% level; Distinct letters in the column indicate significant differences between concentrations according to Tukey's test *(p≤0.05).*

Table 3. Effect of fraction A2 on development of second instar larvae (64-72h old) of *B. cucurbitae.*

Concentrations	Larval Period (Days)	Pupal Period (Days)	Total Developmental Period (Days)	Pupal Weight (mg)	Percent Larval mortality	Percent Pupal mortality
Control	9.32±0.259 ^{a}	10.99±0.378 ^{a}	20.31±0.155 ^{a}	17.67±1.47^{a}	18.33±3.37^{b}	31.67±3.37^{b}
4 ppm	7.93±0.198 ^{b}	7.53±0.401 ^{b}	15.46±0.400 ^{b}	14.33±0.41^{b}	95.17±0.66^{a}	97.67±0.67^{a}
16 ppm	8.33±0.238 ^{ab}	7.07±0.397 ^{b}	15.40±0.200 ^{b}	14.50±0.35^{b}	96.84±0.52^{a}	98.67±0.36 ^{a}
64 ppm	9.03±0.514 ^{ab}	6.70±0.588 ^{b}	15.72±0.324 ^{b}	14.33±0.41^{b}	96.50±0.47^{a}	98.00±0.40 ^{a}
256 ppm	7.81±0.130 ^{b}	7.86±0.232 ^{b}	15.67±0.211 ^{b}	14.50±0.35^{b}	96.50±0.37 ^{b}	97.84±0.44 ^{a}
F- Value	4.897**	17.039**	54.270**	5.780*	587.71**	430.83**

**Significant at 1% level, *Significant at 5% level; Distinct letters in the column indicate significant differences between concentrations according to Tukey's test *(p≤0.05).*

Table 4. Effect of fraction A4 on development of second instar larvae (64-72h old) of *B. cucurbitae*

Concentrations	Larval Period (Days)	Pupal Period (Days)	Total Developmental Period (Days)	Pupal Weight (mg)	Percent Larval mortality	Percent Pupal mortality
Control	9.48±0.272 [a]	11.12±0.294 [a]	20.60±0.144 [a]	16.67±0.82[a]	15.00±2.45[b]	23.34±3.65[b]
4 ppm	9.92±0.271 [a]	8.18±0.593 [b]	18.10±0.455 [b]	14.67±0.41[b]	98.50±0.24[a]	99.00±0.28[a]
16 ppm	10.22±0.557 [a]	7.81±0.397 [b]	18.03±0.280 [b]	14.43±0.29[b]	96.67±1.05[a]	98.50±0.37 [a]
64 ppm	9.63±0.371 [a]	7.95±0.686 [b]	17.58±0.455 [bc]	14.67±0.41[b]	97.67±0.36 [a]	98.34±0.36 [a]
256 ppm	9.25±0.310 [a]	7.06±0.230 [b]	16.31±0.221 [c]	14.33±0.41[b]	98.17±0.44 [a]	98.50±0.37 [a]
F- Value	0.936[NS]	11.080**	25.516**	5.639*	1098.93**	491.47**

**Significant at 1% level, *Significant at 5% level, [NS] Non Significant; Distinct letters in the column indicate significant differences between concentrations according to Tukey's test *(p≤0.05)*

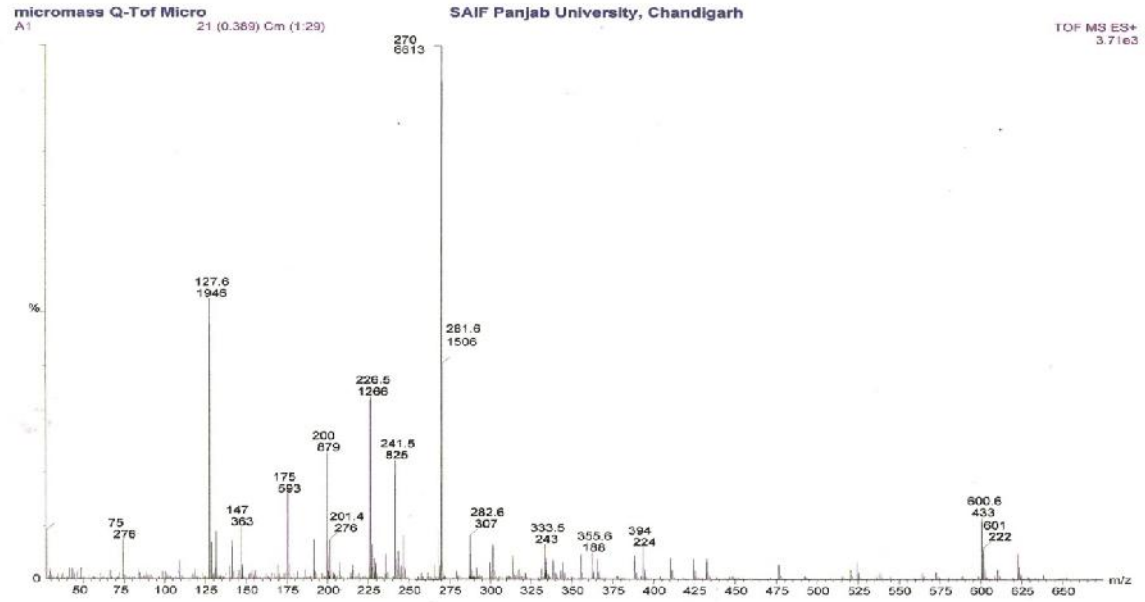

Figure 1. Mass spectrum of major fraction of A1.

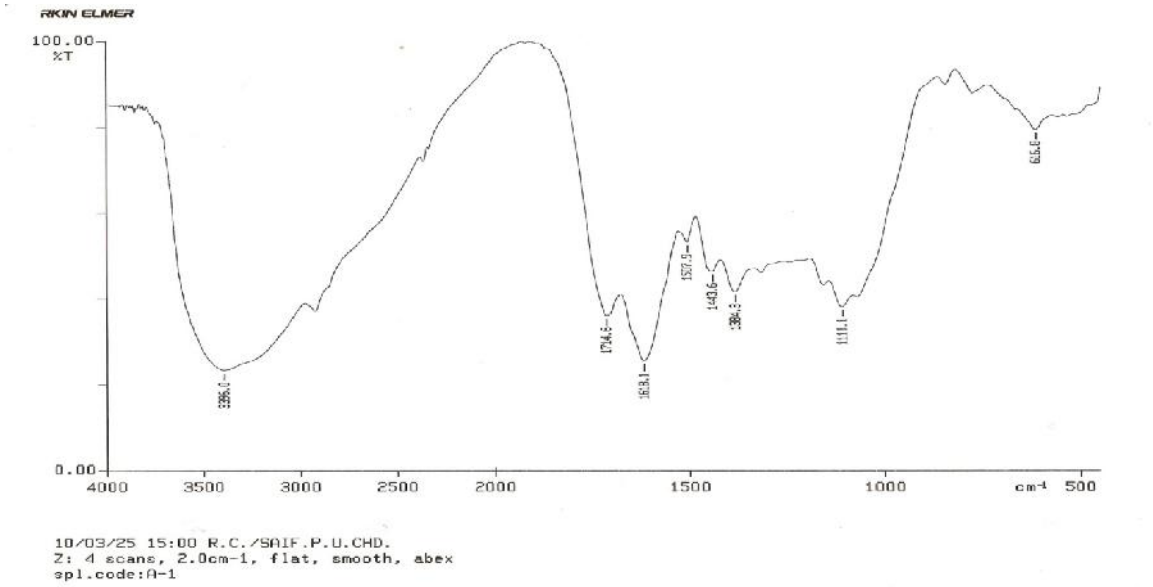

Figure 2. FTIR spectrum of major fraction of A1.

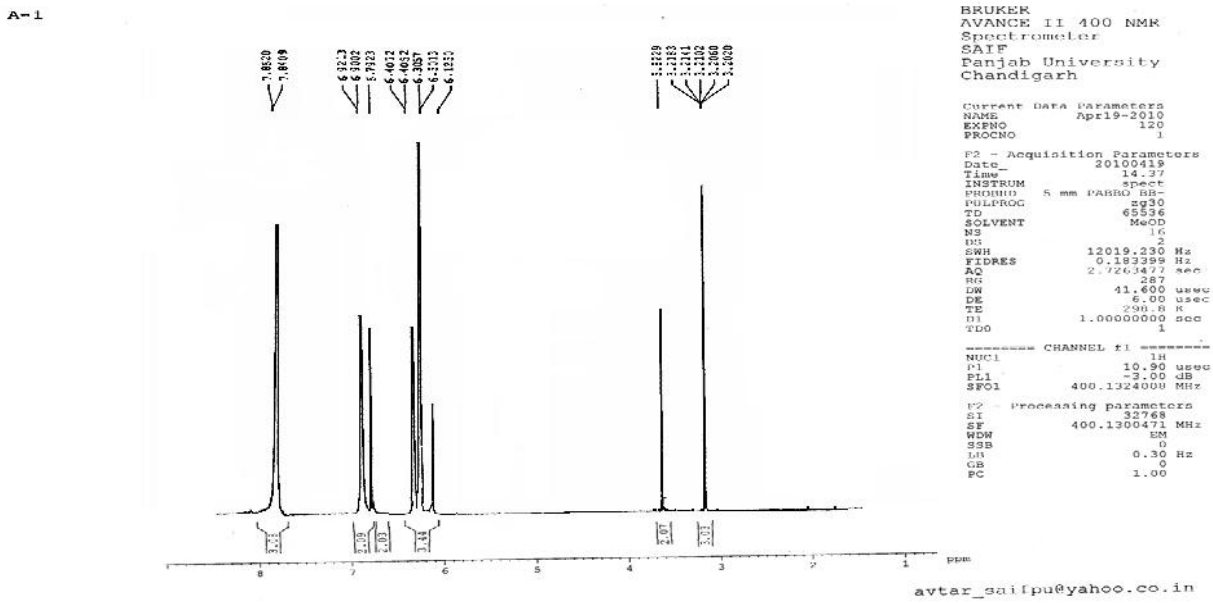

Figure 3. ^{1}H-NMR spectrum major fraction of A1.

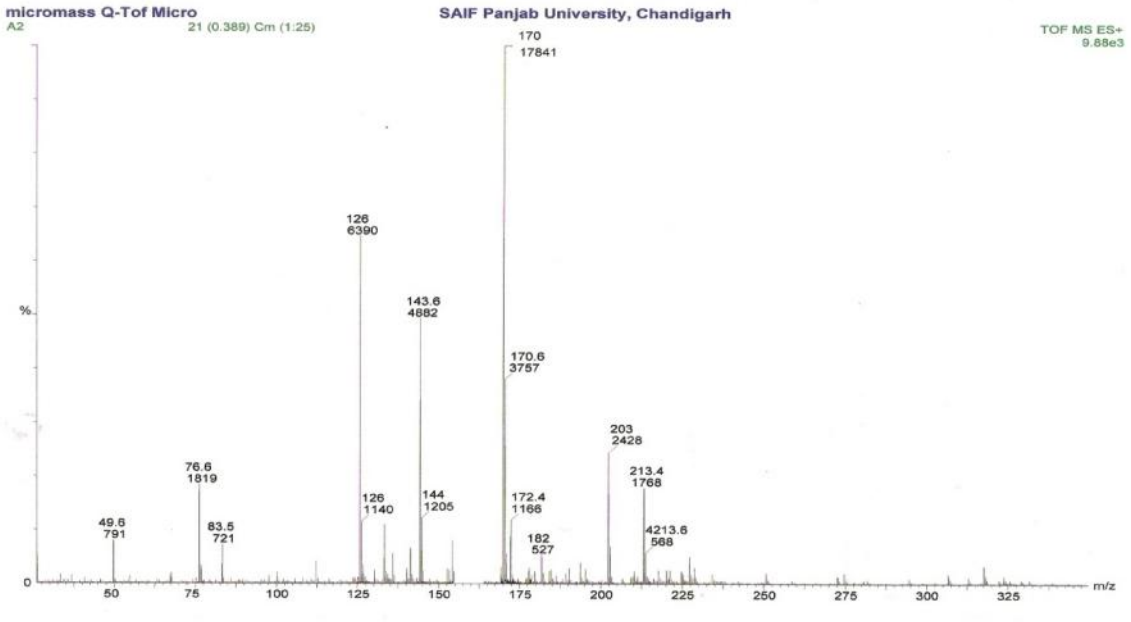

Figure 4. Mass spectrum of major fraction of A2.

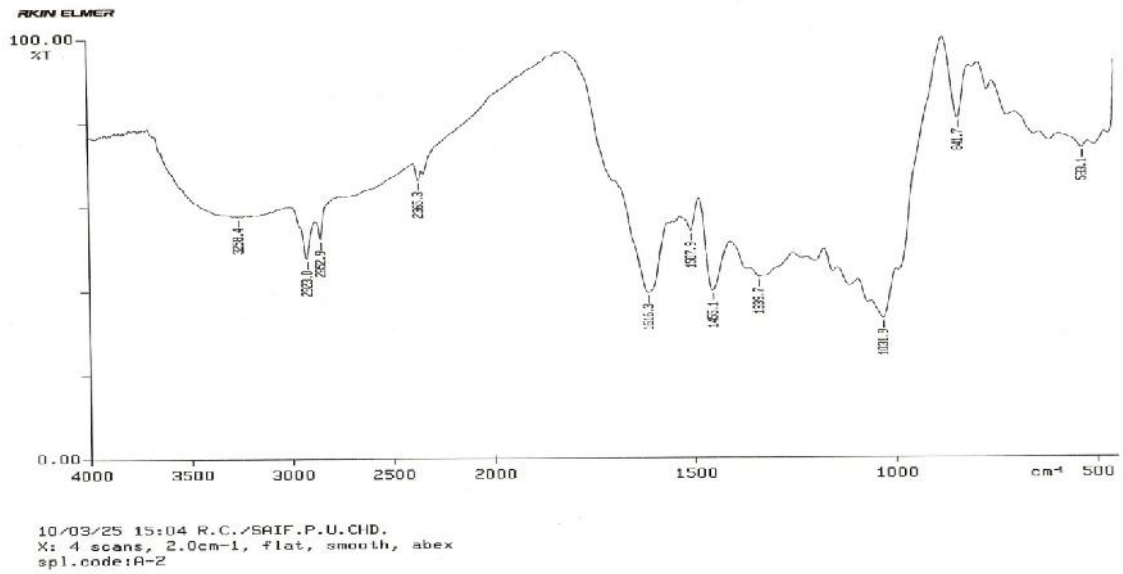

Figure 5. FTIR spectrum of major fraction of A2.

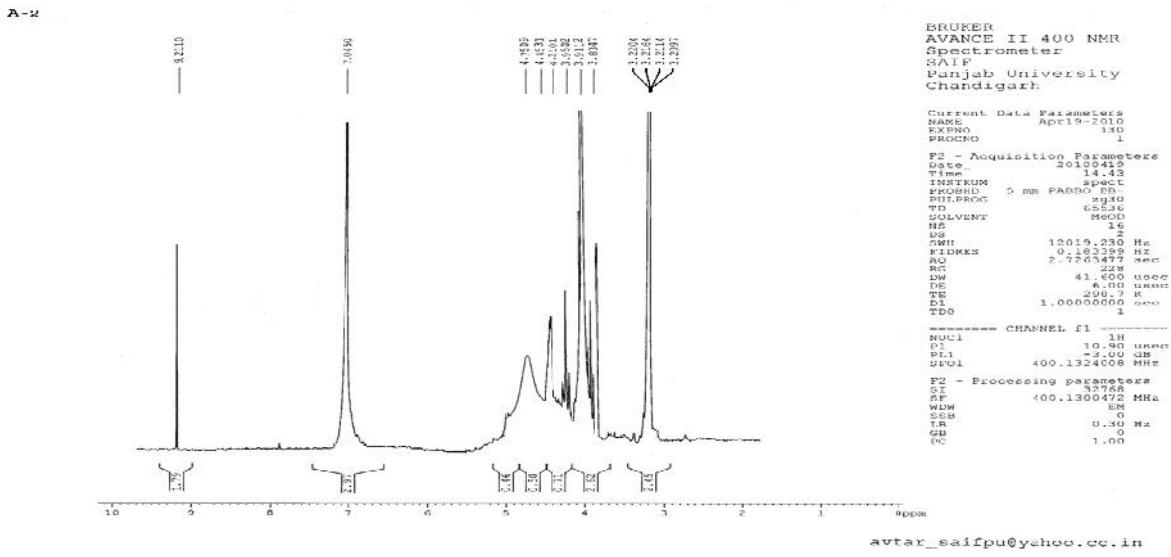

Figure 6. [1]H-NMR spectrum of major fraction of A2.

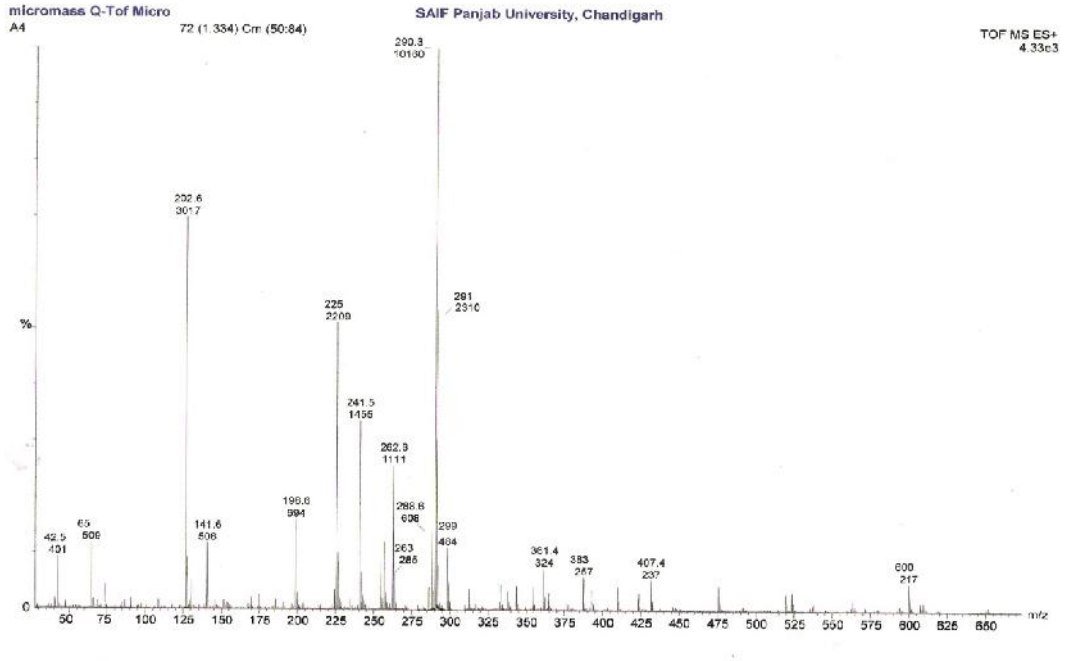

Figure 7. Mass spectrum of major fraction of A4.

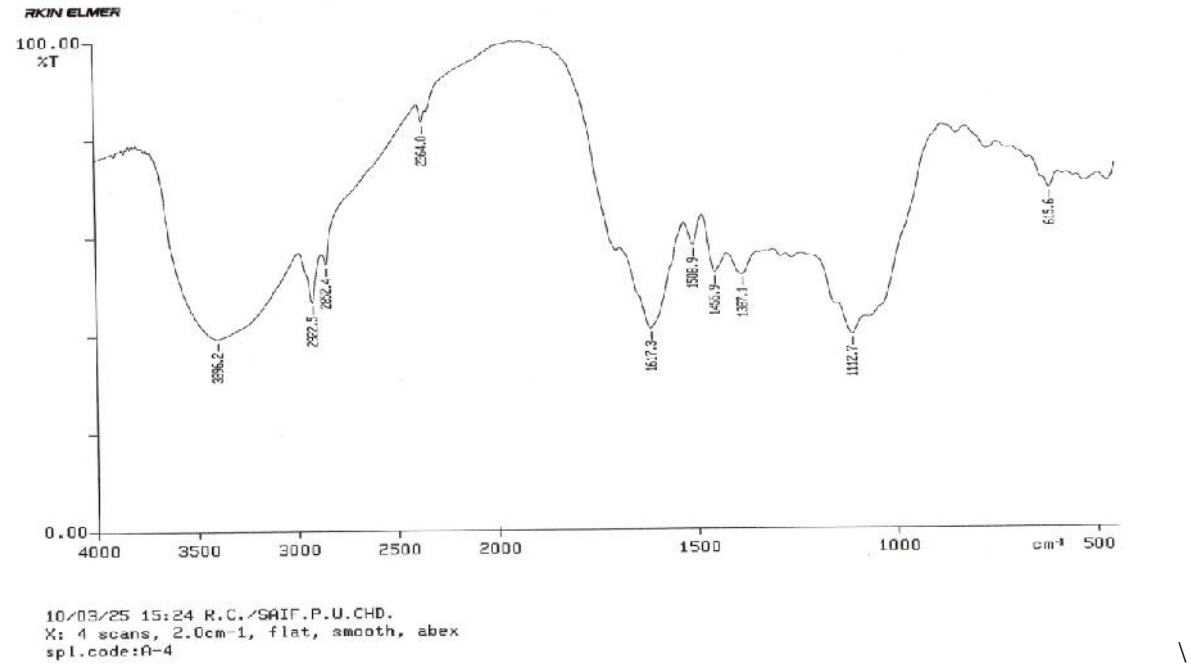

Figure 8. FTIR spectrum of major fraction of A4.

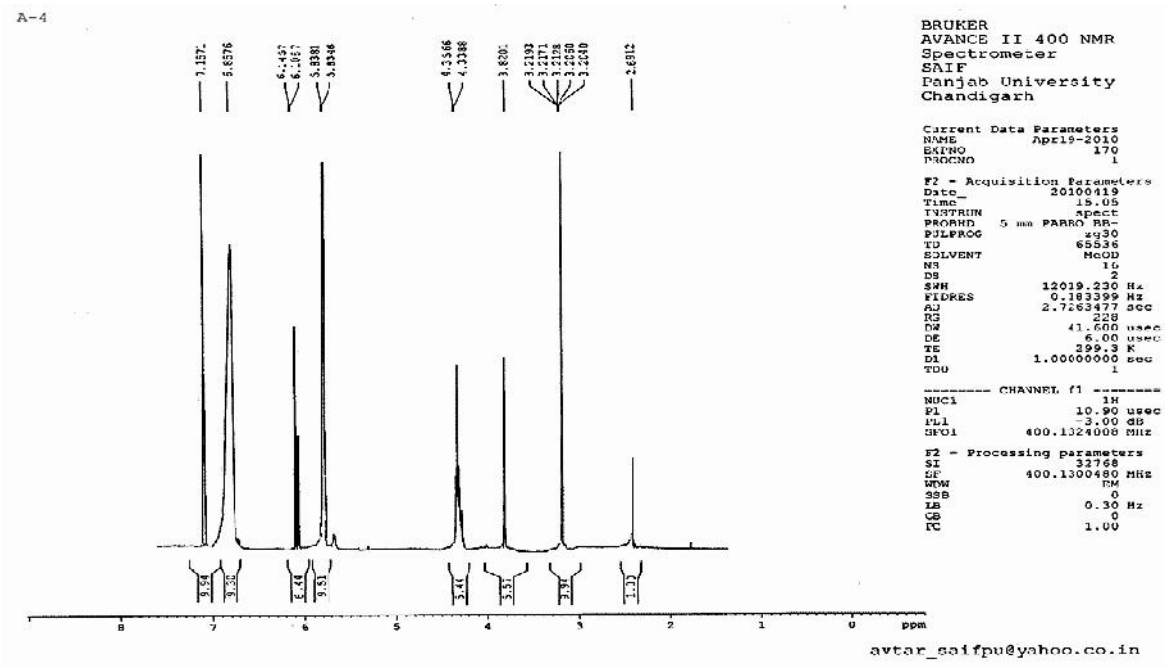

Figure 9. ^{1}H-NMR spectrum of major fraction of A4.

DISCUSSION

Bioassay guided fractionation of plant extracts is one of the most successful methods for discovering new products with biological activity. The column chromatography of the AAE yielded purified fractions which had the flavonoids A1, A2 and A4 as the major active components. In insects, flavonoids interfere with moulting, reproductive and feeding behavior of the insects (Reyes-Chilpa *et al.*, 1995; Musayimana *et al.*, 2001 and Simmonds, 2001) by directly interacting with the steroid hormone system of insects (Oberdorster *et al*., 2001). The bioassays conducted with three fractions viz; fraction A1, A2 and A4 showed an antibiosis effect of the flavonoids against the second instar larvae (64-72h old) of *B. cucurbitae,* consistent with the well established role of the flavonoids in the defense of plants against herbivory (Harborne and Williams, 2000). Insecticidal activity of flavonoids has been reported against the western corn rootworm (Mullin *et al.,* 1992), the common cutworm (Morimoto *et al.,* 2000) and the corn earworm (Widstrom and Snook, 2001).

All the three purified fractions showed varied effects on larval period, but shortened the total development period, decreased pupal weight and increased larval and pupal mortality. The larval period was prolonged with A1 treatment, but was reduced with A2 while A4 had no significant effect. Apigenin and quercetin have been reported to inhibit ecdysone mediated gene transcription thereby altering molting in insects causing death (Oberdorster *et al*., 2001). Antifeedant and growth inhibitory effects of apigenin glycosides have been demonstrated in the pea aphid (Golawska *et al.,* 2010). Apigenin has also been reported to cause high mortality in carrot fly, *Psila rosae* (Fabricius) (Guerin *et al.,* 1983). Apigenin and quercetin along with some other polyphenols have also been implicated in the insecticidal activity of ethanol extracts of *Hybanthus parviflorus* (Mutis ex L. f.) Baill., against *C. capitata* (Broussalis *et al.,* 2010). The larvicidal activity of *Jatropa curcas* L. against malarial vector, *Anopheles arabiensis* Giles was also attributed to the presence of Apigenin along with other flavonoids in the leaves of the plant (Kumar *et al.,* 2008 and Zewdneh *et al.,* 2011). Oliveira *et al.* (2008) showed the presence of five derivative phenolic compounds (mocipinamide, A2, quercetin-3-o-α-L-arabinofuranoside, quercetin and vanicoside D) in the extracts of *Triplaris americana* L., which showed larvicidal activity against *A. aegypti*. Three flavonoids; chlorogenic acid, quercetin and rutin have been identified as components of resistance in the wild species, *A. kempff-mercadoi* against tobacco budworm, *S. litura* (Mallikarjuna *et al.,* 2004). Also among the three flavonoids, statistical analysis had revealed that quercetin had the major effect.

CONCLUSIONS

The active ingredient of *A. auriculiformis* ethyl acetate extract in the form of fractions A1, A2 and A4 proved to be feeding, oviposition and growth deterrent against the second instar larvae of melon fruit fly. The findings also concluded the presence of flavonoids mainly apigenin, gallic acid and catechin in fraction A1, A2 and A4 respectively. This will lead to the formation of base for further development of ecofriendly chemicals from the plant source as well as in the laboratory as potential control agents of insect pests like *B. cucurbitae.*

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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