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GC-MS Analysis and Antifeedant Activity of Azaridiachta indica- Leaf Extract

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ABSTRACT

In the present investigation, the feeding deterrent effects of methanolic and aqueous extracts of *Azaridiachta indica* by using leaf disc with no choice method with some modifications were assessed against cotton whitefly *Bemesia tabaci.* Five different concentrations ranging from 5% - 25 % of each extract were used and their antifeedant effect were recorded after different time periods (24, 48, and 72 hrs.) by comparing the averages of the leaf area consumed in the treated leaves and control leaves. The results clearly decipher that both extracts had antifeedant effects but comparing the extracts, the higher deterrent effect was attained by methanolic extract (87.37%± 12.07) at 25% concentration after 72 hours of post treatment. Antifeedant activity of solvent extracts was assessed based on antifeedant index. Higher antifeedant index normally indicates decreased rate of feeding. The methanolic leaf extract was more effective than that of aqueous solvent. The effect of the extracts was dose dependent and in positive correlation with the concentration. Furthermore, GC-MS based metabolic fingerprinting approach was also employed to find out the composition and relative abundance of active phyto-constituents. It was reported that the chromatogram of methanolic/aqueous extracts of *azadirachta indica* showed 91 and 88 peaks respectively, indicating more number of active constituents using methanol as extraction solvent. The main chemical constituents identified in this study may be responsible for the reported anti-feeding effect of the extract and could offer an alternative source of natural insecticide against *Bemesia tabaci.*

Keywords: *Azadirachta indica;* Deterrent effect; *Bemesia tabaci*; GC-MS analysis; Natural Insecticide

Introduction

The cotton whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is a cosmopolitian, polyphagus and most serious agricultural cotton pest that has caused much heavy losses in productivity of crop, mainly in Fabaceae, Cucurbitaceae and Solanaceae (Oliveira *et al*. 2001) [1]. *Bemisia tabaci is* polyphagous inhabiting more than 600 host plant species. These major sucking insect pests are mainly managed by synthetic insecticides but due of their several adverse effects on environment and human health, plant derivative insecticides are being used by the farmers. At present, plant derived insecticides are well considered as one of the main convenient sources of biorational products with new modes of actions to control phytophagous insects (Rattan, 2010; Dayan *et al*., 2009) [2,3]. Currently more than 46 families of flowering plants are estimated that are known to possess insecticidal properties (Feinstein, 1952) [4]. Amongst these botanicals, neem tree is considered the most promising source for the management of these insect pests (Jacobson, 1988) [5]. It's safe and ecofriendly nature makes it compatible for integrated pest management over the other synthetic insecticides. The control of *B. tabaci* has been taken by chemical insecticides, which dealt with high levels of resistance, damage to non-target organisms and environmental contamination (Elbert and Nauen, 2000) [6]. The hazardous impact of synthetic pesticides on human health and environmental encouraged the use of plant derived pesticides for insect pest management as they are non toxic, easily biodegradable and ecofriendly. A few literatures have dealt with the use of plant derived pesticides or their derivatives as potential bio-pesticides against whiteflies *Bemesia tabaci*. This study amied to screen out the phytochemical constituents in methanolic and aqueous leaf extracts of *Azadirachta indica* by GC-MS analysis and evaluate the antifeedant activity of these extracts agains*t* cotton white fly, *Bemesia tabaci.*

Materials and Methods

Collection of medicinal plant

Selected plant material i.e. leaves of *Azadirachta indica* were collected from different places of Indore region in poly bags and was identified and authenticated at centre for Biodiversity and Taxonomy, University of Kashmir under voucher no. 2248 KASH herb dated 2016. The leaves were shade dried, ground to powder and subjected to extraction in a Soxhlet extraction unit, using methanol and water as extraction solvents. The extraction was done at 30-45°C and finally the extracts were evaporated to dryness using a vacuum evaporator. The dry paste was stored in small vials at -80°C until further use.

Collection and rearing of insects

Adult whiteflies were collected from the cotton field. The stock of colony of *Bemesia tabaci* was maintained on cotton plants in entomological cages (1.2 x 1.2 x 1.0 m) under controlled conditions. The cages were kept in greenhouse at 25- 35°C, 55-75% relative humidity and natural light (12:12h).

Antifeedant Bioassays Defago *et al*., (2006) [7]

The feeding deterrent effects of the *Azadirachta indica* extracts on *Bemesia tabaci* adults starved for 4–5 h prior to each bioassay was determined using leaf disc with no choice method with some modifications. Fresh cotton leaf discs of 1.5 cm in diameter were punched using a cork borer and methanolic and aqueous extracts were applied at different doses (5%, 10%, 15%, 20% and 25%) on both sides of leaf discs individually. Leaf discs treated with water were used as control. After air drying, each leaf disc was placed in petridish containing wet filter paper to avoid early drying of the leaf disc and 5 adults of *Bemesia tabaci* were introduced. For each concentration four replicates were maintained. All the experiments were carried under 18: 6 photoperiods at 20 °C. *Bemesia tabaci* adults were allowed to feed for about 12, 24 and 48 hours and the leaf discs were removed subsequently and the progressive consumption of the leaf disc area in all treatments was recorded using laser leaf area meter (CI- 203CA, CID Inc., WA). Leaf area eaten in each treatment group, was corrected by leaf area eaten in control. The percentage of antifeedant index was calculated using the formula of Jannet *et al*., (2000) [8].

$$AFI = \frac{C - T}{C + T} \times 100$$

Where AFI = Antifeedant Index;

C = Area protected in control leaf disc;

T = Area protected in treated leaf disc

Gas chromatography – Mass spectrometry (GC-MS) analysis

Metabolomic fingerprinting of methanolic and aqueous extracts of *Azadirachta indica*-leaves was carried out as described Roessner *et al.,* (2000) [9] with some modifications. Both the alcoholic and aqueous extracts were dried and resuspended in methanol and filtered through 0.45µ syringe filter. About 2µl of each sample was injected in a GC-MS AP2010 Plus system (Shimadzu, Japan) equipped with a programmable head space auto injector/sampler and a Flame thermionic detector (FTD). The capillary used was DB- 1/RTXMS-1 (30 m) with helium gas as carrier at a constant flow rate of 1.21 ml/min. The samples were injected in a split less mode at an injection temperature of 260 °C/ column oven temperature of 60°C. The temperature gradient applied to GC oven, during the analysis was at 60 °C/ 2 minutes; then 250 °C at a rate of 5°C/minute for 2 minutes followed by a temperature ramp of 300 °C at a rate of 15 °C/minute for 15 minutes. The system was set at an ion source temperature of 220 °C with an interface temperature of 270 °C; detector gain volume at 0.00kV and the solvent cut time of 4.5 minutes in a relative gain mode. Mass spectra were recorded between 5.0-60.32 min. of injection in an ACQ scanning mode; event time of 0.5 sec/ scanning speed 1250 in the m/z range of 50-650. Identification of individual components was achieved by comparing the retention times and molecular masses of individual peaks from GC with those from the Wiley and National Institute of Standards and Technology (NIST) Library. The GC-MS was carried out at Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University (JNU), New Delhi.

Results

The results presented in Figures 1a and b indicate that both the methanolic and aqueous extracts of *Azadirachta indica* showed the antifeedent activity. Antifeedant property of solvent extracts was examined mainly by antifeedant index. Maximum antifeedant index revealed minimum amount of feeding. The maximum antifeedant activity at 72 hours of treatment was shown by methanolic extract 87.37%± 12.07 at 25% concentration while as at 5.0 % concentration the respective antifeedant value was 80.44%±13.21 at $P<0.05$. The corresponding antifeedant value of aqueous extracts at 25% concentration was 83.86±15.12. While at 5% concentration the respective antifeedant value was 74.93%±8.65 at $P<0.05$. Similarly, the maximum antifeedant activity at 48 hours of treatment was shown by methanolic extract 58.14%±7.43 at 25% concentration while as at 5% concentration the respective antifeedant value was 53.63%±7.23 at $P<0.05$. The corresponding antifeedant activities of respective aqueous extract at 25% concentration was 54.71%±6.23. While as 5% concentration the respective antifeedant values was 47.11%±7.21 at $P<0.0$. Similarly, the maximum antifeedant activity at 24 hours of treatment was shown by methanolic extract (40.90%±5.07) at 25% concentration while as at 5% concentration the respective antifeedant value was 36.67%±2.55 at $P<0.05$. The corresponding antifeedant activitiy of respective aqueous extract at 25% concentration was 39.69%±9.35. While as 5% concentration the respective antifeedant values was 30.74%±8.65 at $P<0.05$.

Above mentioned results clearly decipher that both the methanolic as well aqueous extracts of *Azadirachta indica* showed antifeedant activitiy at all concentrations and time durations of treatment, but comparing the extracts, methanolic extracts showed the maximum percentage of antifeedant activitiy at 25 % or 5% concentrations after 72 hours of post treatment while as aqueous extract showed the lowest percentage of antifeedant activitiy 25 % or 5% concentration after 24 hours of duration.

Thus the result illustrates that the antifeedant potential of extracts towards the pest was in a dose dependent manner-- the higher the concentration the greater the antifeedant activity and vice versa. However the effect seemed dependent on time of exposure as well.

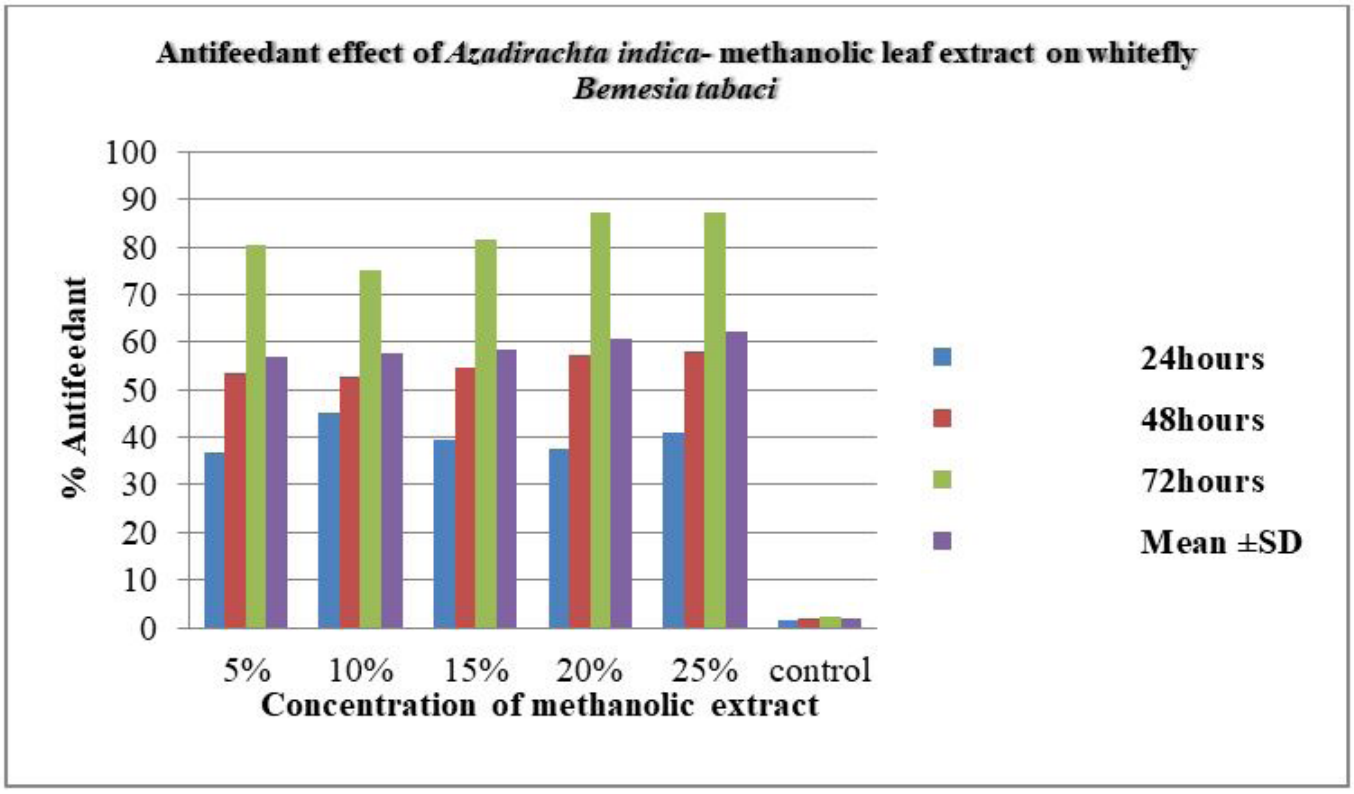

Figure 1: (a) Graph showing percentages of antifeedant property of methanolic *Azadirachta indica*-leaf extract on cotton whitefly *Bemesia tabaci*

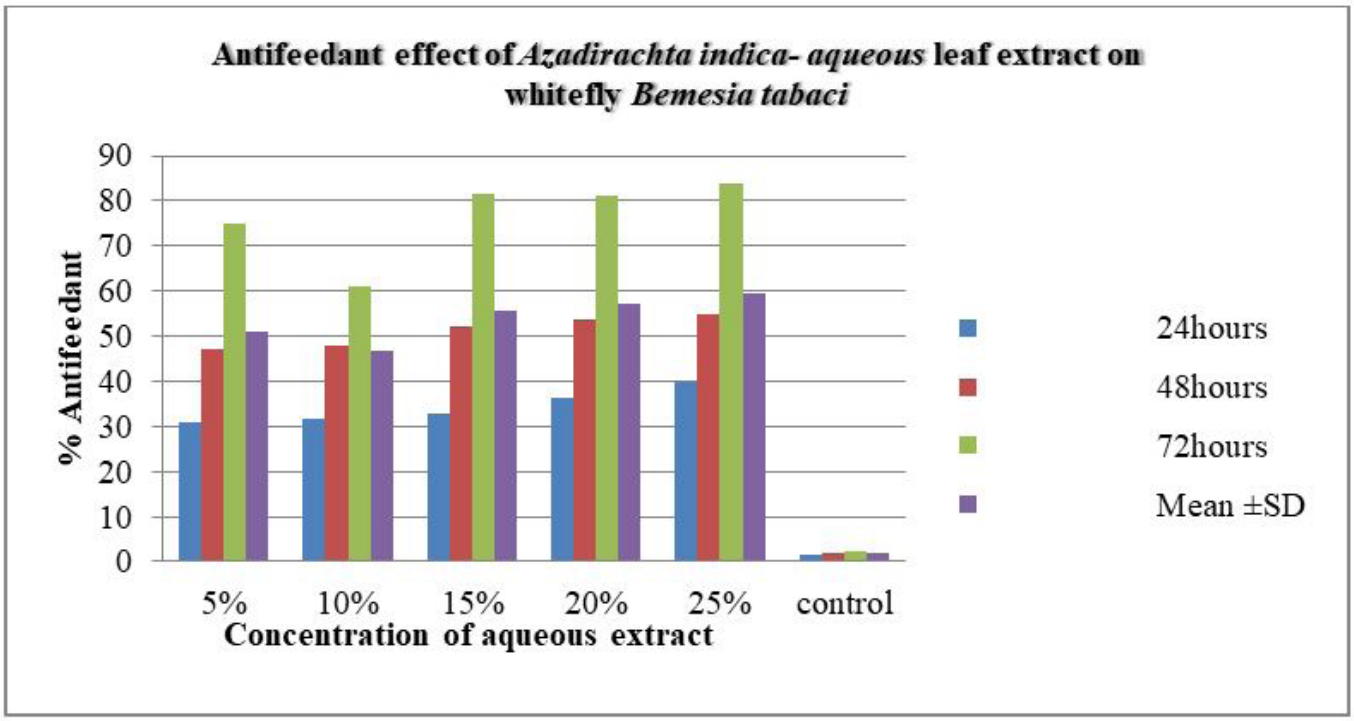

Figure 1: (b) Graph showing percentages of antifeedant property of aqueous *Azadirachta indica*-leaf extract on cotton whitefly *Bemesia tabaci*

Gas chromatography- Mass spectrometry (GC-MS) analysis

To find out the composition and relative abundance of active phyto-constituents; using aqueous or alcoholic solvents and its correlation with the insecticidal activity, GC-MS based metabolic fingerprinting approach was employed. The chromatogram of methanolic/aqueous extracts of *Azadirachta indica* is represented in Figure 2a and b showing 91 and 88 peaks respectively, indicating more number of active constituents using methanol as extraction solvent. The constituents present in the methanolic/aqueous extracts of *Azadirachta indica,* corresponding to the chromatogram peaks along with their retention time (RT), percent peak area and the identified name from NIST- WILEY library are shown in Table 1a and b. It is clear from the table that the most abundant constituents- 15 & 12 from each extract (in terms of percent peak area) lie in the range of 1-25%, constituting 87.60% and 83.04% of total percent peak area of aqueous and methanolic extracts, respectively. To compare the insecticidal activity and hence efficiency of two extraction solvents, it was further analyzed the abundance of component/s in different class intervals of percent peak areas. The biological activity of each predominant compound is also shown (Table 2a and b), reflecting their bioactivities and benefits.

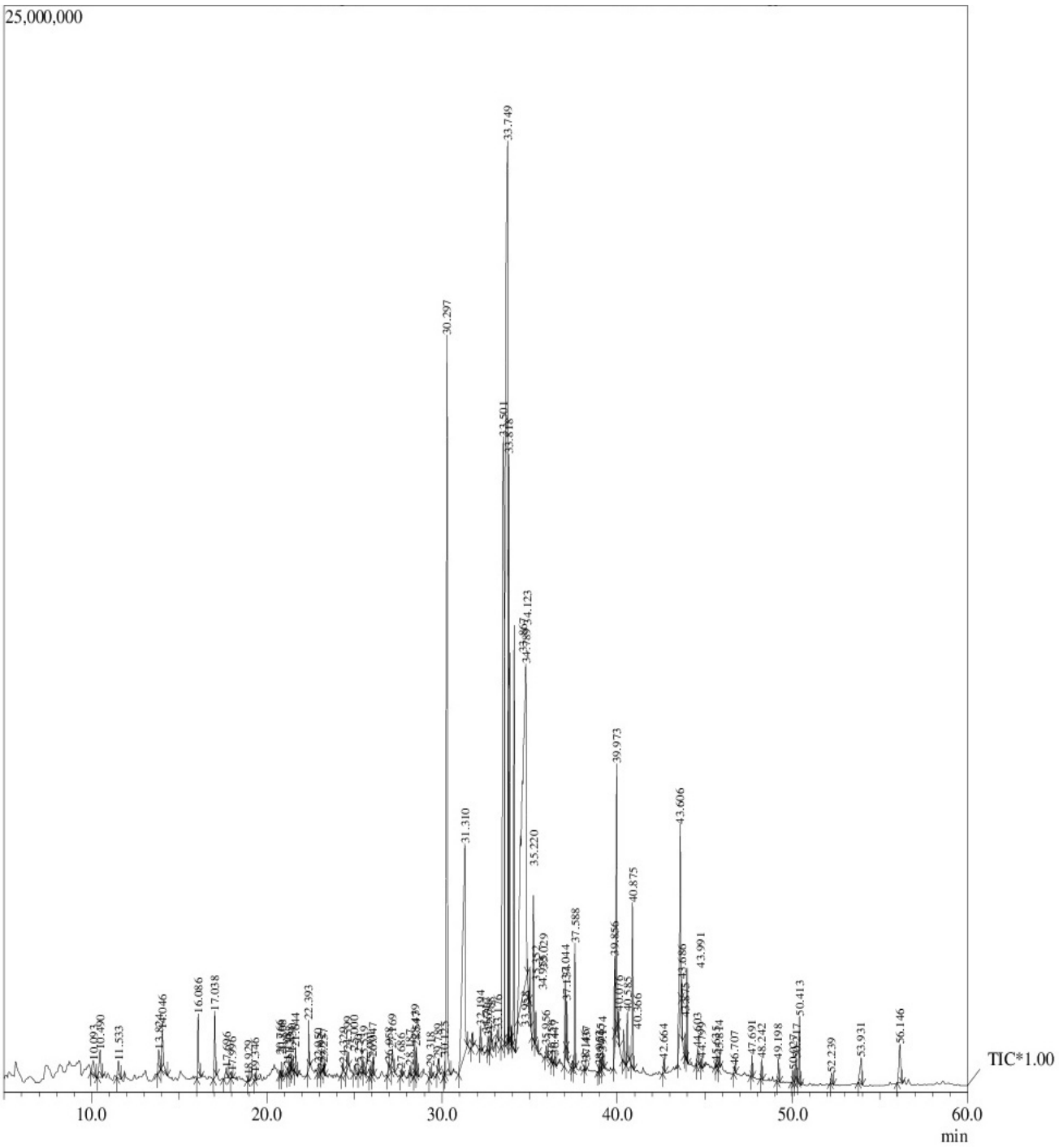

Figure 2: (a) GC- MS chromatogram of methanolic rhizome extracts of *Zingiber officinale*

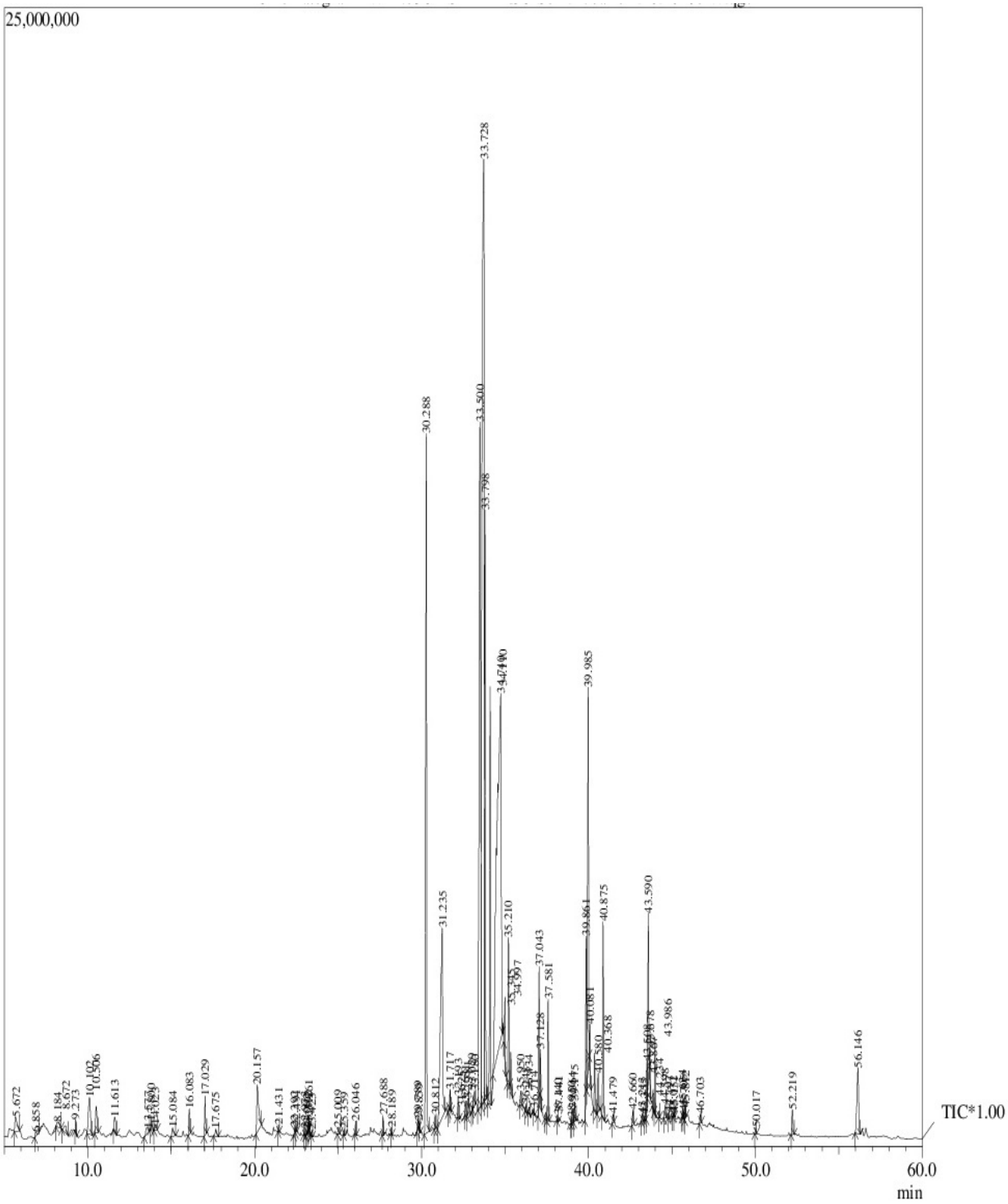

Figure 2: (b) GC- MS chromatogram of aqueous leaf extracts of *Azadirachta indica*

Peak No.	Retention time	Area%	Compound Name
1	5.672	0.38	2(3h)-Furanone, 5-Methyl-
2	6.858	0.05	
3	8.184	0.16	3-Methylcyclopentane-1,2-Dione
4	8.672	0.19	Glycerin
5	9.273	0.08	Silane, Methoxytrimethyl-
6	10.102	0.88	Diazene, Bis(1,1-Dimethylethyl)-
7	10.506	0.42	4h-Pyran-4-One, 3-Hydroxy-2-Methyl-
8	11.613	0.26	6-Octenal, 3,7-dimethyl-, (R)-
9	13.577	0.16	
10	13.800	0.12	2,3-Dihydro-Benzofuran
11	14.023	0.12	5-Hydroxymethylfurfural
12	15.084	0.17	Ethanone, 1-(2,5-Dihydroxyphenyl)-
13	16.083	0.25	2-Methoxy-4-vinylphenol
14	17.029	0.40	Phenol, 2,6-dimethoxy-
15	17.675	0.12	3,3-Dimethylglutaric acid
16	20.157	1.03	Benzoic Acid, 4-Hydroxy-, Methyl Ester
17	21.431	0.05	Dodecanoic Acid, Methyl Ester
18	22.392	0.05	Methyl-3-methoxy-5-methyl benzoate
19	22.491	0.05	Dodecanoic Acid
20	22.973	0.05	1,2-Benzenedicarboxylic Acid, Diethyl Ester
21	23.092	0.03	
22	23.192	0.03	2-Hydroxyethyl salicylate
23	23.261	0.09	Hexadecane
24	23.423	0.04	
25	25.009	0.09	8-Hexadecenal, 14-methyl-, (Z)-
26	25.359	0.04	2-Propenoic acid, tridecyl ester
27	26.046	0.09	Methyl tetradecanoate
28	27.688	0.14	Iron, Tricarbonyl[N-(Phenyl-2-Pyridinylmethylene)Benzenamine-N,N
29	28.189	0.05	Pentadecanoic acid, methyl ester
30	29.789	0.10	9-Hexadecenoic Acid, Methyl Ester, (Z)-
31	29.858	0.04	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
32	30.288	7.83	Hexadecanoic acid, methyl ester
33	30.812	0.11	Dibutyl phthalate
34	31.235	4.74	n-Hexadecanoic acid
35	31.625	0.02	13-Tetradecenal
36	31.717	0.15	2-Methyltetracosane
37	32.193	0.18	Heptadecanoic acid, methyl ester
38	32.631	0.14	9-Octadecenoic acid
39	32.781	0.18	17-Octadecen-14-ynoic acid, methyl ester
40	33.029	0.04	9,12-Octadecadienoic acid (Z,Z)-
41	33.180	0.21	2-[12-(2-Oxiranyl)Dodecyl]Oxirane
42	33.500	12.38	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
43	33.728	22.96	Phthalic Acid
44	33.798	5.02	9,12-Octadecadienoic acid, methyl ester
45	34.110	3.77	Methyl stearate
46	34.740	17.18	cis-Vaccenic acid

47	34.997	0.60	Octadecanoic acid
48	35.210	1.33	11,14-Octadecadienoic acid, methyl ester
49	35.345	0.33	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl
50	35.950	0.16	9,12-Octadecadienoic acid (Z,Z)-
51	36.248	0.05	2-Piperidinone, N-[4-bromo-n-butyl]-
52	36.434	0.05	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclo
53	36.714	0.03	13-Octadecenal, (Z)-
54	37.043	1.09	Glycidylpalmitate
55	37.128	0.34	Methyl 9-eicosenoate
56	37.441	0.08	Methyl 5,11,14-eicosatrienoate
57	37.581	0.93	Eicosanoic Acid, Methyl Ester
58	38.140	0.04	9-Octadecenamide, (Z)-
59	38.950	0.04	9,12-Octadecadienoyl chloride, (Z,Z)-
60	39.064	0.13	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester
61	39.175	0.21	(E)-13-Docosenoic acid
62	39.861	1.48	9,12-Octadecadienoyl chloride, (Z,Z)-
63	39.985	4.08	Glycidyloleate
64	40.081	0.33	9,12-Octadecadienoyl chloride, (Z,Z)-
65	40.368	0.60	Glycidylpalmitate
66	40.580	0.40	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
67	40.875	1.89	Triacontanoic acid, methyl ester
68	40.479	0.08	1,E-8,Z-10-Hexadecatriene
69	42.660	0.13	
70	43.213	0.06	Oleic acid, 3-hydroxypropyl ester (Z)-
71	43.335	0.03	9-Octadecen-1-Ol
72	43.508	0.34	9,12-Octadecadienoyl chloride, (Z,Z)-
73	43.590	1.58	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-
74	43.678	0.21	7-Tetradecenal(Z)-
75	43.867	0.12	Octadecanoic acid, 2,3-dihydroxypropyl ester
76	43.986	0.49	Tetracosanoic Acid, Methyl Ester
77	44.234	0.03	Maltose 8tms
78	44.598	0.15	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-
79	44.797	0.03	Squalene
80	45.072	0.02	Hexadecanoic Acid, 1-(1-Methylethyl)-1,2-Ethanediyl Ester
81	45.151	0.02	Hexopyranose, 1,2,3,4,6-Pentakis-O-(Trimethylsilyl)-
82	45.634	0.05	Glycidylpalmitate
83	45.705	0.04	Levoglucosan, 3TMS derivative
84	45.812	0.06	Hexacosanoic acid, methyl ester
85	46.703	0.08	Cyclooctanecarboxaldehyde
86	50.017	0.05	beta.-Sitosterol
87	52.219	0.31	9-Octadecenal, (Z)-

Table 1a: Phytocomponents identified in the aqueous leaf extracts of *Azadirachta indica* by GC- MS analysis

Peak No.	Retention time	Area%	Compound Name
1	10.093	0.31	Diazene, Bis(1,1-Dimethylethyl)
2	10.490	0.51	4-(4-Methyl-Piperazin-1-Yl)-1,5,-Dihydro-Imidazol-2-One
3	11.533	0.29	1,5-Anhydro-6-Deoxyhexo-2,3-Diulose
4	13.824	0.38	2,3-Dihydro-Benzofuran
5	14.046	0.71	4-Hydroxy-2-Methyl-Pyrrolidine-2-Carboxylic Acid
6	16.086	0.66	2-Methoxy-4-Vinylphenol
7	17.038	0.65	Phenol, 2,6-Dimethoxy-
8	17.696	0.18	3,3-Dimethylglutaric Acid
9	17.996	0.05	Cyclobuta[1,2:3,4]Dicyclopentene, 1,2,3,3a,3b.Beta.,4,5,6,6a.Beta.,6b.Alph
10	18.929	0.04	Bicyclo[7.2.0]Undec-4-Ene, 4,11,11-Trimethyl-8-Methylene-, [1r-(1r*,4e,9s
11	19.346	0.05	1-Phenethyl-4-Acetoxypiperidine
12	20.766	0.04	(1S,5S)-2-Methyl-5-((R)-6-Methylhept-5-En-2-Yl)Bicyclo[3.1.0]Hex-2-Ene
13	20.868	0.04	Octadecane, 1-Chloro-
14	21.198	0.04	1,3-Cyclopentanedione, 4-Methyl-5-Pentyl-
15	21.338	0.08	1,8(2H,5H)-Naphthalenedione, Hexahydro-8a-Methyl-, Cis-
16	21.430	0.07	Dodecanoic Acid, Methyl Ester
17	21.644	0.19	2(4H)-Benzofuranone, 5,6,7,7a-Tetrahydro-4,4,7a-Trimethyl-, (R)-
18	22.393	0.42	3',5'-Dimethoxyacetophenone
19	22.950	0.12	
20	23.092	0.04	4-Vinylbicyclo[3.3.1]Nonane-2,7-Dione
21	23.257	0.05	Hexadecane
22	24.329	0.09	Pyrrolidine, 1-(1-Cyclohexen-1-Yl)-
23	24.599	0.20	4-(6,6-Dimethyl-2-Methylene-3-Cyclohexen-1-Ylidene)-2-Pentanol
24	25.000	0.09	E-7-Octadecene
25	25.250	0.06	1-{2-[3-(2-Acetyl-Oxiran-2-Yl)-1,1-Dimethyl-Propyl]-Cycloprop-2-Enyl}
26	25.539	0.13	
27	25.901	0.08	1,1,4,7-Tetramethyldecahydro-1H Cyclopropa[E]Azulene-4,7-Diol
28	26.047	0.12	Methyl Tetradecanoate
29	26.958	0.09	
30	27.169	0.33	2(4h)-Benzofuranone, 5,6,7,7a-Tetrahydro-6-Hydroxy-4,4,7a-Trimethyl
31	27.686	0.05	Octadecane
32	28.187	0.07	Pentadecanoic Acid, Methyl Ester
33	28.439	0.27	Neophytadiene
34	28.547	0.23	2-Pentadecanone
35	29.318	0.10	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol
36	29.789	0.14	9-Hexadecenoic Acid, Methyl Ester, (Z)-
37	30.125	0.04	9-Hexadecenoic Acid, Methyl Ester, (Z)-
38	30.297	8.56	Hexadecanoic Acid, Methyl Ester
39	31.310	6.00	N-Hexadecanoic Acid
40	31.717	0.11	Batilol
41	32.194	0.21	Heptadecanoic Acid, Methyl Ester
42	32.631	0.13	11-Dodecyn-1-Ol Acetate
43	32.788	0.18	17-Octadecen-14-Ynoic Acid, Methyl Ester
44	33.176	0.17	2-[12-(2-Oxiranyl)Dodecyl]Oxirane
45	33.501	12.98	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester
46	33.749	22.86	9-Octadecenoic Acid, Methyl Ester, (E)-
47	33.818	4.65	9,12-Octadecadienoic Acid, Methyl Ester

48	33.876	1.99	Trans-Phytol
49	33.958	0.06	
50	34.123	3.71	Methyl Stearate
51	34.789	15.14	Cis-Vaccenic Acid
52	34.955	0.03	
53	35.029	0.30	Octadecanoic Acid
54	35.220	1.32	11,14-Octadecadienoic Acid, Methyl Ester
55	35.352	0.33	Cyclopropanebutanoic Acid, 2-[[2-[[2-[(2-Pentylcyclopropyl)Methyl]Cyclopropyl] Methyl]Cyclo
56	35.956	0.12	9,12-Octadecadienoic Acid
57	36.256	0.04	1,1,1-Trifluoroheptadecen-2-One
58	36.447	0.08	Hexadecadienoic Acid, Methyl Ester
59	37.044	0.61	Glycidyl Palmitate
60	37.134	0.34	Methyl 9-Eicosenoate
61	37.437	0.07	3,6-Octadecadienoic Acid, Methyl Ester
62	37.588	0.94	Eicosanoic Acid, Methyl Ester
63	38.146	0.03	9-Octadecenamide, (Z)-
64	38.952	0.05	9,12-Octadecadienoyl Chloride, (Z,Z)-
65	39.065	0.10	9,12-Octadecadienoyl Chloride, (Z,Z)-
66	39.174	0.17	(E)-13-Docosenoic Acid
67	39.856	0.68	9,12-Octadecadienoyl Chloride, (Z,Z)-
68	39.973	2.35	Glycidyl Oleate
69	40.076	0.14	9,12-Octadecadienoyl Chloride, (Z,Z)-
70	40.366	0.38	Glycidyl Palmitate
71	40.585	0.50	Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester
72	40.875	1.33	Docosanoic Acid, Methyl Ester
73	42.664	0.11	Tricosanoic Acid, Methyl Ester
74	43.606	2.15	9-Octadecenoic Acid, 1,2,3-Propanetriyl Ester, (E,E,E)-
75	43.686	0.17	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl
76	43.875	0.15	Octadecanoic acid, 2,3-dihydroxypropyl ester
77	43.991	0.52	Tetracosanoic acid, methyl ester
78	44.603	0.19	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-
79	44.799	0.05	Squalene
80	45.635	0.02	Glycidyl palmitate
81	45.814	0.08	Triacontanoic acid, methyl ester
82	46.707	0.06	Nonanol, Trimethyl-
83	47.691	0.14	Vitamin E
84	48.242	0.15	Andrographolide
85	49.198	0.21	Stigmasterol
86	50.027	0.11	beta.-Sitosterol
87	50.217	0.25	Retinoic acid
88	50.413	0.65	Diazoprogesterone
89	52.239	0.13	8-Hexadecenal, 14-methyl-, (Z)-
90	53.931	0.44	Retinoic acid
91	56.146	0.71	13-Octadecenal, (Z)-

Table 1b: Phytocomponents identified in the methanolic leaf extracts of *Azadirachta indica* by GC- MS analysis

Class Interval	Compound name	Retention time	Biological activity
1-2%	Benzoic Acid, 4-Hydroxy-, Methyl Ester	20.157	Antimicrobial (Duke ,2013)
	11,14-Octadecadienoic acid, methyl ester	35.210	Antioxidant(Devi *et al.,* 2012); anti-inflammatory, hypo-cholesterolemic, 5-alpha reductase inhibitor, nematicide, **pesticide** and anti-androgenic (Praveen *et al.*,2010; Duke, 2013;Aleryani *et al.,* 2005)
	Glycidyl palmitate	37.043	Larvicidal (Sivakumar *et al.,* 2011); Nematicide, **pesticide**(Duke ,2013,) Anti-cancer properties (Biljana; 2012)
	9,12-Octadecadienoyl chloride, (Z,Z)-	39.861	Nematicide,Hepatoprotective,Antiandrogenic, Antihistaminic, Anticoronary, **Insectifuge**, Antieczemic, Anticancer (Duke ,2013)
	Triacontanoic acid, methyl ester	40.875	Nematicide, anticancer, anti-inflammatory, **insectifuge** (Duke, 2013,)
	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	43.590	Cytotoxic and anti-proliferative activity (Kuppuswamy *et al.,* 2013); Antimicrobial (Gehan *et al.,* 2009)
	E,E-2,13 Octadecadien-1-ol	56.146	Anti-inflammatory and antioxidant (Duke, 2013) (Gopalakrishnan and Kalaiarasi 2013)
>2-5%	n-Hexadecanoic acid	31.235	Antioxidant, antibacterial, nematicide, antiinflammatory, hypocholesterolemic, **pesticide**, lubricant, antiandrogenic, antitumor, flavour, cancer preventive, immunostimulant, chemopreventive, haemolytic 5-α reductase inhibitor, lipooxygenase inhibitor. (Kapoor and Huang, 2006; Galli and Calder, 2009)
	Methyl stearate	34.110	Antidiarrheal (Suresh *et al .,* 2014) and cytotoxic and antiproliferative Activities (Kuppuswamy *et al .,* 2013)
	Glycidyl oleate	39.985	Anti-inflammatory, hypo-cholesterolemic and anti-arthritic (Rani *et al.,* 2009)
>5-10%	Hexadecanoic acid, methyl ester	30.288	Anticancer (Willits *et al.,*1952)
	9,12-Octadecadienoic acid, methyl ester	33.798	Hypocholesterolemic, Nematicide Antiarthritic, Hepatoprotective Anti androgenic, Hypocholesterolemic Nematicide, 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary **Insectifuge**, Antieczemic, Antiacne Anticancerous and diuretic (Mathew (2011).
>10-20%	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	33.500	Antibecterial activty (Lima *et al.*, (2011)
	cis-Vaccenic acid	34.740	Anti-inflammatory and antioxidant compounds (Duke ,2013,)
>20-25%	33.728	33.728	

Table 2a: Reported biological activities of the identified bioactive compounds from the aqueous extracts of the *Azadirachta indica*

Class interval	Compound name	Retention time	Biological activity
1-2%	2-Hexadecen-1-ol	33.876	No activity reported
	11,14-Octadecadienoic acid, methyl ester	35.220	No activity reported
	Docosanoic Acid, Methyl Ester	40.875	Antioxidants and anti peptic ulcer agent (Dauda 2017)
>2-5%	9,12-Octadecadienoic acid, methyl ester	33.818	Nematicide, anticancer, anti-inflammatory, **insectifuge**(Duke,2013)
	Methyl stearate	34.123	Cytotoxic and anti-proliferative activity (Kuppuswamy *et al.*,2013)
	Glycidyl oleate	39.973	Anticancer (Willits *et al.*,1952)
	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	43.606	No activity reported
>5-10%	Hexadecanoic acid, methyl ester	30.297	Antioxidant(Devi *et al* 2012); anti-inflammatory, hypo-cholesterolemic, 5-alpha reductase inhibitor, nematicide, **pesticide** and anti-androgenic (Praveen *et al.*,2010; Duke, 2013;Aleryani *et al.,* 2005)
	n-Hexadecanoic acid	31.310	Larvicidial activity(Sivakumar *et al.,* 2011), nematicide, **pesticide** (Duke JA ,2013)
>10-20%	cis-Vaccenic acid	34.789	Anti-inflammatory and antioxidant compounds (Duke , 2013; Gopalakrishnan and Kalaiarasi 2013)
	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	33.501	Anti-inflammatory, hypo-cholesterolemic, cancer preventive, hepatoprotective, nematicide, **insectifuge**, anti-histaminic, anti-arthritic, anti-coronary, and anti- androgenic (Duke , 2013)
>20-25%	9-Octadecenoic acid, methyl ester, (E)-	33.749	Anti-histaminic, hepatoprotective, hypo-cholesterolemic and anti-eczemic (Duke ,2013)

Table 2b: Reported biological activities of the identified bioactive compounds from the methanolic extracts of the *Azadirachta indica*

Discussion

Azadirachta indica derivatives showed more reduction of the insect pest population. This is mostly due its deterrent and antifeedant effect which compell whiteflies to fly away from that locality. Khattak *et al.,* (2000) [10] investigated that the detrimental effect of 1000ppm neem oil treatment lost by 30 days after treatment but the 10,000ppm treatment effectively retained its antifeedant and deterrent effects against maize weevil on corn kernels. Khan *et al.,* (2002) [11] also demonstrated that due to the antifeedant and deterrent effect of *Azadirachta indica* extracts, the populations of jassids, thrips and whiteflies on cotton significantly reduced 17 days after spray.. Silva (2007) [12] investigated the antifeedant properties of the hydroalcoholic extract obtained from the leaves of *Azadirachta indica* on *Zabrotes fasciatus* (Coleoptera: Bruchidae), an insect pest that commonly feeds on common bean (*Phaseolus vulgaris)* during seed storage and observed the significant antifeedant activity when it was added to the insect diet. Alice Sujeetha (2008) [13] showed that on rice, extracts of neem seeds and neem leaves inhibit the growth and development of *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae). These results are in agreement with the present investigation.

Previously the ethyl acetate extract of leaf of *Azadirachta indica* has been shown to contain a total of 30 volatile compounds with Hexadecanoic acid; 9/10 Octadecanoic acid methyl ester; methyl stearate; cis 11- Eicosanoic acid; Docasonoic acid methyl ester as major constituents Praveen *et al.*, (2018) [14]. In compliance to this report, our study reports a total of 91 and 88 peaks from methanolic and aqueous extracts of neem leaf respectively, corresponding to 12 and 15 major constituents/ peaks. In addition to this, out of total 30 peaks from fruit sap/pulp of neem, Hexadecanoic and Pentadecanoic acids were the major peaks/ fatty acids Kumar *et*

al., (2018) [15]. Further a study reported only 4 peaks from methanolic fraction of neem leaf comprising- m-Toluyl-aldehyde; Methyl 14-methylpentadecanoate; Linoleic acid chloride and Methyl isoheptadecanoate while they were comparing different solvent systems with the richness of chromatogram produced Hossain *et al.,* (2013) [16].

To sum up, it is quite evident that the approach of selection of water and methanol as extraction solvents yielded a significant higher proportion of bioactive components comprising predominantly of fatty acids or their esters with diversifying activities. However, to validate the lead insecticidal molecules further studies are needed to perform bioactivity based fractionation as well as characterization of active molecules. This could help to synthesize the active lead insecticidal molecules in the lab with better efficacies and least side effects thereby preventing the plant wealth and to maintain nature's diversity without disturbing the ecological balance of the planet [17,31].

Conclusion

According to the results obtained in the current study, it can be concluded that both methanolic and aqueous extracts of *Azadirachta indica* presented a high insecticidal activity against *B. tabaci* and showed positive relationship with concentration. The results of this study raises the possibility that the insecticidal properties of the active compound(s) present in the tested plant extracts could be exploited as an alternate of many synthetic chemical insecticides being indiscriminately used for control of *B. tabaci.*

Conflicts of interest

We declare no conflict/s of interest related to this work.

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