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# Identification of Volatile Constituents and Biological Activity Potential of *Abelia triflora*

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**Abstract:** *Abelia triflora*, a member of the plant family i.e., Caprifoliaceae, is known for its pharmaceutical properties including antioxidant, antibacterial, anti-inflammatory, and anticancer benefits. An experiment was performed to investigate the insecticidal and antibiotic potential of *A. triflora*. It is widely distributed under diverse ecological conditions including America and Eastern Asia. Main objectives of this study were to explore the biological activities of *A. triflora* and describe its chemical constituents. Two plant extracts i.e., ethyl acetate and n-hexane, were isolated from the *A. triflora* plants and tested for their insecticidal and antibacterial characteristics. Moreover, the n-hexane fraction was analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS) to recognize its chemical constituents. The GC MS analysis identified 36 phytochemicals like fatty acids, phenols, stigmasterols, and vitamins, often linked with various biological activities. The insecticidal activity tests of n-hexane extract were promising and demonstrated strong efficacy (100% inhibition) against *Sitophilus oryzae* (rice weevil), a notorious grain pest. However, the extract was not effective in controlling other investigated insect species like *Callosobruchus analis*, *Rhyzopertha dominicia*, *Tribolium castaneum*, and *Trogoderma granarium*. On the other hand, ethyl acetate fraction of *A. triflora* was ineffective against the tested insect species. Moreover, the ethyl acetate fraction showed moderate inhibition (46.57%) of *Staphylococcus aureus*, a common bacterium. The n-hexane fraction, however, showed no antibacterial activity. These findings suggest phytochemical potential of *A. triflora* against insects and bacteria. Further studies may help to explore its full potential for developing medicines or natural pest control solutions.

**Keywords**: GC MS, bactericidal activity, insecticidal activity, Phytochemicals, biological pest control, natural insecticides, *Abelia triflora*.

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## 1. Introduction

Natural products, derived from animals, microorganisms and plants, have a long history in traditional and modern medicines. They also continue to be a vital resource for modern drug discovery (Gautam and Dwivedi, 2022; Krause and Tobin. 2013). These complex biomolecules are enriched with diverse biological activities, making them instrumental in the development of new medicines and serve as a tool for understanding biological systems (Bernardini et al., 2018; Sun and Shahrajabian, 2023). Importantly, secondary metabolites, are often produced by plants in response to stress, and play a vital role in the organism's defense mechanisms and chemical signals for communication with other organisms (Al-Khayri et al., 2023; Khare et al., 2020; Qaderi et al., 2023).

Chemistry of natural products primarily emphasizes biosynthesis, biological functions, and chemical aspects of secondary metabolites, mainly due to their unique features and potentially diverse applications especially in medicine, agriculture and certain industries (Satish et al., 2020; Twaij et al., 2022).

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Numerous pharmaceuticals, cosmeceuticals, and nutraceuticals utilize natural compounds as their active ingredients. Plants, animals and microorganisms are the most important sources of active natural compounds used for drug synthesis and discovery (Chávez-Arias et al., 2022; Guan et al., 2021; Sharma et al., 2022).

Medicinal plants are rich source of therapeutic phytochemicals, having a long history for the treatment of various ailments (Fitzgerald et al., 2020). They remained an integral component of primary healthcare for the treatment of different diseases within the different communities of the world, especially in underdeveloped and rural areas (Ayati et al., 2020; Zhou et al., 2013). In addition to the treatment of human ailments, medicinal plants are also vital in the treatment of animal diseases (Hossen et al., 2022; Shirsath and Goswami, 2020; Sofowora et al., 2013).

*Abelia triflora* is a member of the honeysuckle family (Caprifoliaceae). This family comprises 42 genera and 862 species and is mainly distributed in Eurasia, North America, Mexico, and Eastern Asia (Xu and Chang, 2017). Members of the genus *Abelia* are mostly shrubs and vines (Acharya and Mukherjee. 2017; Ghimire et al., 2018). This family contains dicotyledonous flowering plants, opposite leaves and berry or drupe fruits (Landrein et al., 2012). Species are generally diploid and self-incompatible (Scobie and Wilcock. 2009; Xu and Chang, 2017 ).

The Abelia genus is native to China and primarily consists of ornamental shrubs. Five species and 11 genotypes are mostly found in East Asia and the Himalayas (Cornara et al., 2021; Landrein et al., 2017 ). Out of 80 species of the genus *Abelia* only *A. triflora* is present in Pakistan, which is a shrub native to East Asia and the Himalayas (Chrząszcz et al., 2021; Landrein and Farjon, 2019). This plant is mostly known for its persistent sepals, tubular corolla and trilocular capsule (Cornara et al., 2021; Villarreal-Quintanilla et al., 2013).

In the recent years gas chromatography (GC) and mass spectrometry (MS) have been widely used as a basic technology to study the secondary metabolite in both plant and non-plant species. Despite the therapeutical potential of *A. triflora*, limited research is available in the literature to describe its phytochemical profile (Fawzy et al., 2017). Therefore, a study was designed to characterize phytochemicals extracted from *A. triflora* using ethyl acetate and n-hexane. Moreover, to evaluate the insecticidal and antibacterial potential of its extract fractions.

## 2. Materials and Methods

### 2.1. Plant Material

Plants of *A. triflora* were collected from the Ziarat Valley, Balochistan, Pakistan. After species verification (by Dr. Rasool Bakhsh Tareen, Professor, Department of Botany, University of Balochistan, Quetta, Pakistan) leaves were subjected to phytochemical extraction, thrice, using methanol. Further methanolic extract was fractionated into ethyl acetate and n-hexane fractions.

### 2.2. GC-MS Analysis

GC-MS analysis was performed to identify the chemical constituents of different fractions. Moreover, retention time was determined by GC, while molecular and fragment ion peaks were determined by MS. For GC analysis the column (30m × 250µm × 0.25µm) was filled with 5% phenylmethyl siloxane and was used as stationary phase and helium gas was used as mobile phase. The sample was passed along the column and was allowed by the mobile phase. For MS analysis the oven temperature was adjusted to 50 °C (three minutes) and subsequently, the temperature was increased to 300°C. The highest noted pressure and temperature of the oven were 9.05 psi and 360°C respectively. After a total run of 68 minutes, the sample mass spectra were obtained, which were compared with the existing libraries in Manlib and Raplib. Electron impact (EI) was applied as a source of ionization at 250 °C (Enerijiofi et al., 2021; Kanthal, et al., 2014).

### 2.3. Insecticidal Activity

The minimum inhibitory concentration (MIC) of both samples (n-hexane and ethyl acetate fractions) was calculated by microdilution method. Five bacterial species were selected from gram-negative [*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Salmonella typhi* (ATCC 14028)] and gram-positive [*Bacillus subtilis* (ATCC 23857), *Staphylococcus auereus* (NCTC 6571)] bacteria for antibacterial activity.

Hexane and ethyl acetate fractions were dissolved in dimethyl sulfoxide (DMSO) solvent and again diluted (2.0 to 0.312 mg/mL) in the Muller Hinton broth containing all mentioned bacteria. For growth control, pure DMSO was used. All experiments were performed in duplicates while those microdilution plates were incubated at 36 °C (for 18h). Bacterial growth was monitored and confirmed when yellow color was changed into purple color in the well mixture, after the addition of 20µL alcoholic solution to each well (Balouiri, et al., 2016).

### 2.4. Antibacterial Activity

Five insect species including [*Callosbruchus analis* (Cowpea beetle), *Tribolium custeneum* (Flour beetle), *Trogoderma granarium* (Khapra beetle), *Sitophilus oryzae* (Rice weevil), *Rhyzopertha dominica* (Lesser grain borer)] were selected to evaluate insecticidal activity of ethyl acetate and n-hexane fractions of plant extracts of *A. triflora*. The mass cultures of each of these insects were maintained in 1000 ml glass jars with relevant food material (19:1, whole wheat flour and dried yeast). Moreover, subcultures were kept in smaller beakers (500ml) for easy processing of the experiment. During the experiments, laboratory temperature was maintained at 30±0.5 °C.

**Table 1. Retention time, compound name, formula, molecular weight of phytochemicals present in n-hexane fraction of *Abelia triflora* obtained through GC-MS**

| Peak No. | RT (min) | Name of Compounds | Mol. Formula | Mol. Weight | Area Sum% |
|---|---|---|---|---|---|
| 1 | 11.86 | 2,4-pentadien-1-ol,3-propyl-, | $C_8H_{14}O$ | 126 | 0.1 |
| 2 | 12.71 | 9-Oxabicyclo[6.1.0]nonan-4-ol | $C_8H_{14}O_2$ | 142 | 0.14 |
| 3 | 15.32 | Nonanoic acid | $C_9H_{18}O_2$ | 158 | 0.08 |
| 4 | 15.63 | Phenol,2-methyl-5-(1-methylethyl)- | $C_{10}H_{14}O$ | 150 | 0.12 |
| 5 | 16.09 | 2-Methoxy-4-vinylphenol | $C_9H_{10}O_2$ | 150 | 0.05 |
| 6 | 23.62 | Asarone | $C_{12}H_{16}O_3$ | 208 | 0.14 |
| 7 | 23.69 | Apiol | $C_{12}H_{14}O_4$ | 222 | 0.25 |
| 8 | 26.58 | 2-Propenoic acid,3-(4-methoxy phenyl)-,ethyl ester | $C_{12}H_{14}O_3$ | 206 | 0.61 |
| 9 | 28.18 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | 296 | 0.26 |
| 10 | 28.33 | [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester | $C_{21}H_{38}O_2$ | 322 | 0.16 |
| 11 | 29.96 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270 | 1.09 |
| 12 | 31.63 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 21.19 |
| 13 | 33.61 | 9,12-Octadecadienoic acid(Z,Z)-,methyl ester | $C_{19}H_{34}O_2$ | 294 | 0.84 |
| 14 | 33.81 | 9,Octadecenoic acid(Z)-,methyl ester | $C_{19}H_{36}O_2$ | 296 | 1.18 |
| 15 | 37.06 | 9,12-Octadecadienoic acid(Z,Z)- | $C_{18}H_{32}O_2$ | 280 | 37.96 |
| 16 | 37.41 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 0.73 |
| 17 | 54.94 | Behenic alchol | $C_{22}H_{46}O$ | 326 | 2.08 |
| 18 | 59.06 | 1,2-Benzenedicarboxylic acid,diisooctyl ester | $C_{24}H_{38}O_4$ | 390 | 4.04 |
| 19 | 62.54 | 7-Methyl-Z-tetradecen-1-ol acetate | $C_{17}H_{32}O_2$ | 268 | 0.26 |
| 20 | 64.87 | 9,12-Octadecadienoyl chloride,(Z,Z) | $C_{18}H_{31}ClO$ | 298 | 2.12 |
| 21 | 65.82 | Erucic acid | $C_{22}H_{42}O_2$ | 338 | 0.75 |
| 22 | 68.01 | Ethyl iso-allocholate | $C_{26}H_{44}O_5$ | 436 | 0.87 |
| 23 | 66.27 | 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-acridinedione | $C_{19}H_{13}BrClNO_3$ | 417 | 0.95 |
| 24 | 68.72 | 5,14,23-Octadecatrien-14,15-diol | $C_{28}H_{52}O_2$ | 420 | 1.22 |
| 25 | 69.29 | 1-Hexadecanol,2-methyl- | $C_{17}H_{36}O$ | 256 | 0.42 |
| 26 | 71.88 | Cholesta-4-6,dien-3-ol,(3β)- | $C_{27}H_{44}O$ | 384 | 2.84 |
| 27 | 72.59 | Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy-,pivalate | $C_{33}H_{54}O_3$ | 498 | 0.46 |
| 28 | 73.07 | Vitamin E | $C_{29}H_{50}O_2$ | 430 | 0.38 |
| 29 | 75.77 | B-Sistosterol | $C_{29}H_{50}O$ | 414 | 7.45 |
| 30 | 76.12 | 9,19-Cyclolanost-24-en-3-ol,acetate,(3β)- | $C_{32}H_{52}O_2$ | 468 | 0.57 |
| 31 | 76.58 | Lupeol | $C_{30}H_{50}O$ | 426 | 1.12 |
| 32 | 76.94 | Stigmasta-3,5-dien-7-one | $C_{29}H_{46}O$ | 410 | 3.48 |
| 33 | 77.47 | Stigmast-4-en-3-one | $C_{29}H_{48}O$ | 412 | 2.98 |
| 34 | 78.09 | 1-Heptatriacotanol | $C_{37}H_{76}O$ | 536 | 0.48 |
| 35 | 79.56 | Betulin | $C_{30}H_{50}O_2$ | 442 | 1.13 |
| 36 | 80.11 | Stigmastane-3,6-dione,(5α) | $C_{29}H_{48}O_2$ | 428 | 0.56 |

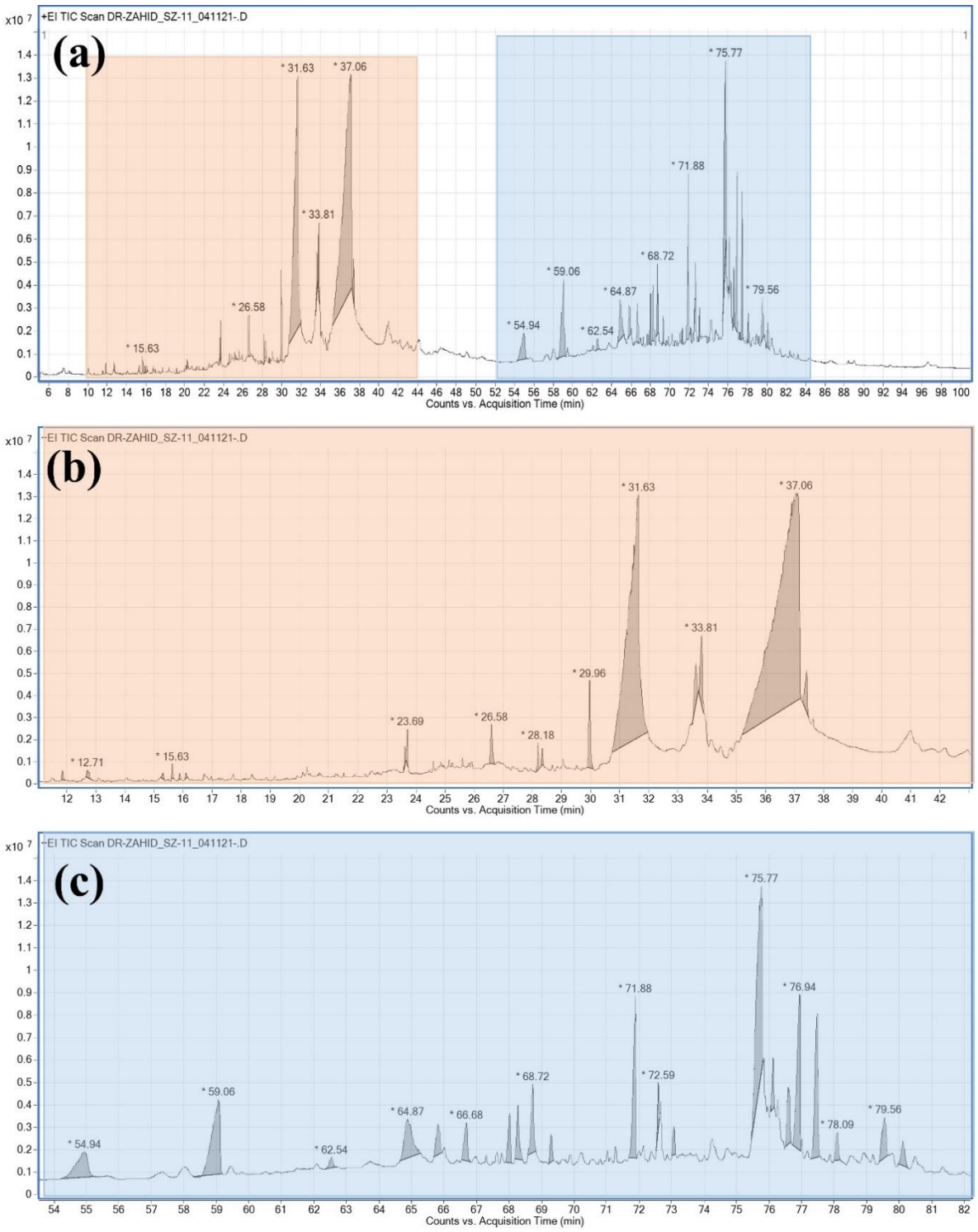


**Fig. 1. GC-MS chromatogram of n-hexane fraction of *Abelia triflora***

## 2.5. Mortality Test

The mortality of the adults of the *Tribolium custeneum* was evaluated by film residual method, after 24, 48 and 72h of the treatment. Abott's formula of mortality rate was employed, as described in Eq [1].

$$Mc = (Mo-Mc/100-Me) \times 100 \quad [1]$$

Where, Mo: Observed mortality rate (%); Me: mortality control rate (%); MC: is Corrected mortality rate (%).

## 3. Results and Discussion

### 3.1. GC-MS Analysis

GC-MS analysis was done on the n-hexane fraction of *A. triflora* extract to recognize its chemical composition. Identified phytochemicals are listed, in the Table 1, along with their molecular formula (MF), molecular weight (MW), % peak area and retention time (RT). Chromatograms obtained from GC represent the association between retention time (RT) and relative concentration of each phytochemical (Fig. 1). The secondary metabolites present in the extract influence the insecticidal and antimicrobial activity in the test (Fig. 2).

The bioactive compounds present in the extract were identified based on the height of the chromatogram peaks (Table 1, Fig. 1). Moreover, to confirm the identity of phytochemicals obtained, mass spectra of each phytochemical were obtained using MS analysis (Fig. 3). The results showed that n-hexane fraction of *A. triflora* contains 36 phytochemicals including Nonanoic acid, Lupeol (antifungal potential), Asarone, n-hexanoic acid, Octadecanoic acid, β-sitosterol and Betulin (antibacterial potential), and Vitamin E and Behenyl alchol (good potential for the human skincare). The presence of such diverse bioactive phytochemicals in the n-hexane fraction confirms the potential medicinal importance of *A. triflora* (Al Hashmi et al., 2013; Konappa et al., 2020; Shang et al., 2011).

### 3.2. Insecticidal Activity

The insecticidal potential of both fractions (ethyl acetate and n-hexane) of *A. triflora* was assessed during analysis. The insecticidal activity was determined against five insects (*Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominicia*, *Collobruchus analis* and *Trogoderma granarium*) by using the film residual method.

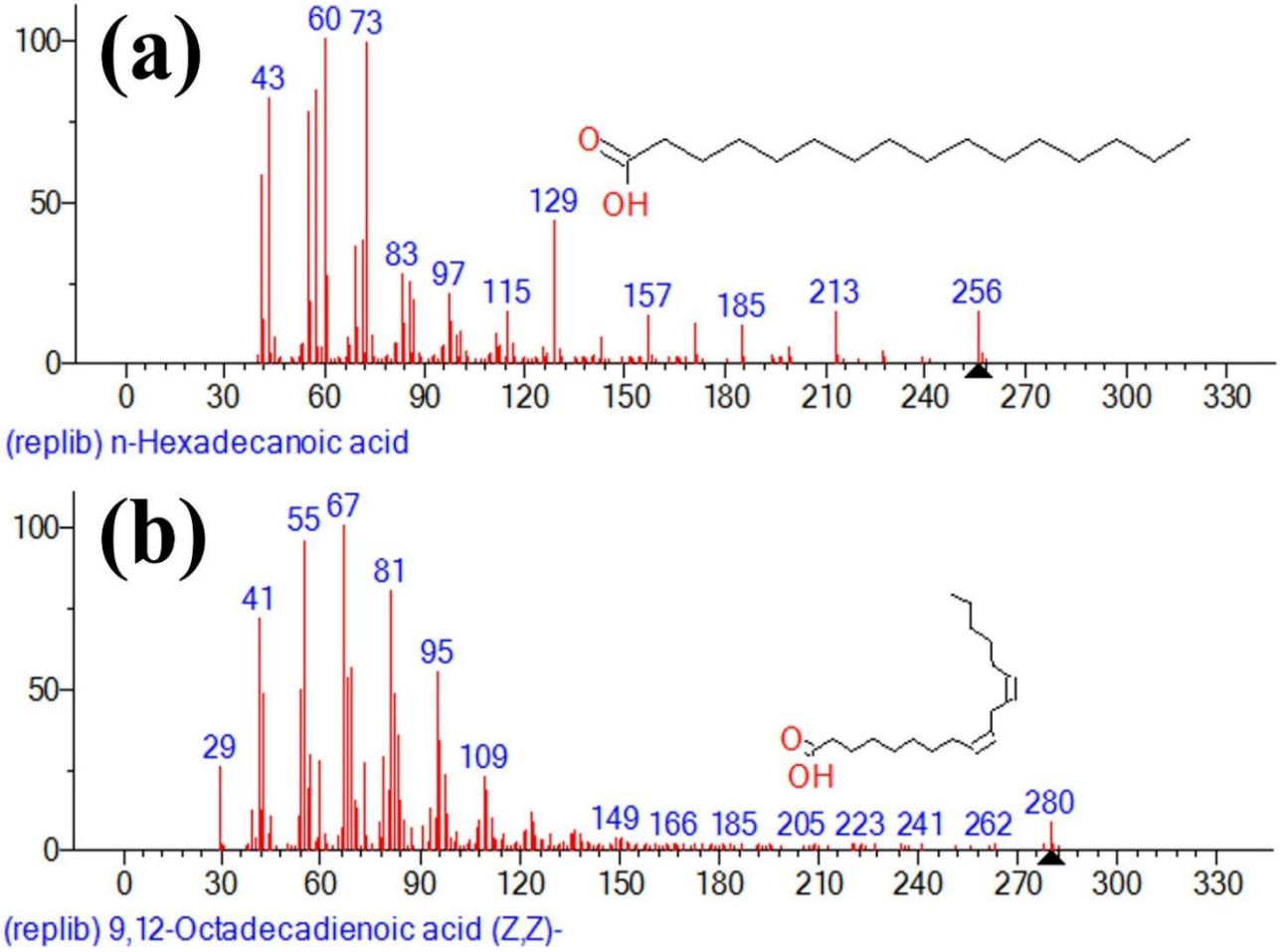


**Fig. 2. Mass spectrum and structural compound of (a) n-Hexadecanoic acid, (b) 9,12-Octadecanoic acid(Z,Z) obtained from n-hexane fraction of *Abelia triflora*.**

**Table 2. Percentage inhibition of insect species by different fractions of *Abelia triflora* extracts**

| Insect species | n-hexane fraction | Ethyl acetate fraction | Pumethum |
|---|---|---|---|
| *S. oryzae* | 100% | 0 | 100% |
| *R. dominicia* | 0 | 3.45 | 0 |

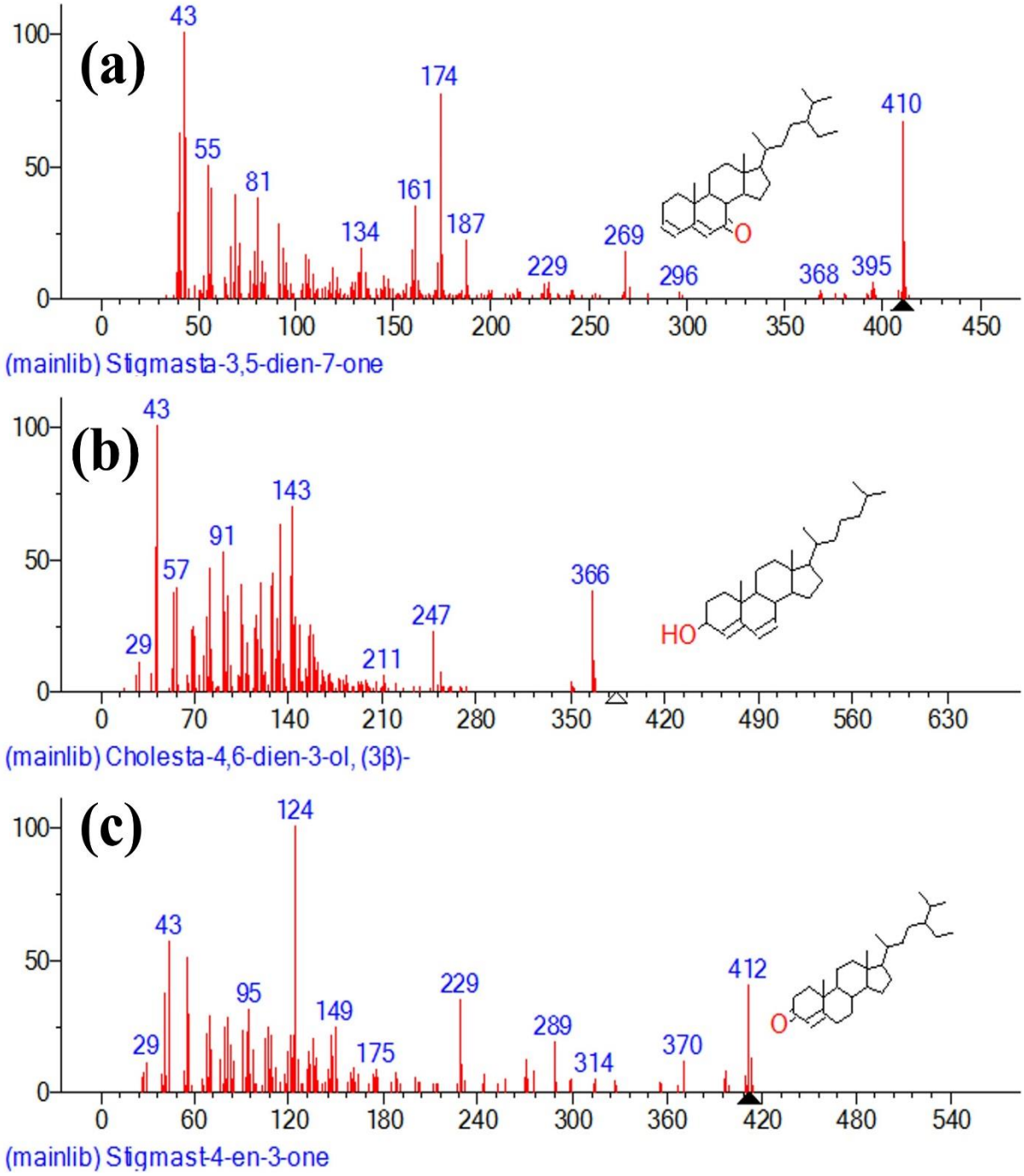


**Fig. 3. Mass spectrum and structural compound of (a) Stigmasta-3,5-dien-7-one, (b) Cholesta-4,6-dien-3-ol,(3β), (c) Stigmasta-4-en-3-one obtained from n-hexane fraction of *Abelia triflora***

**Table 3. Percentage inhibition of bacterial species by different fractions of *Abelia triflora* extracts**

| Bacterial species | n-hexane fraction | Ethyl acetate fraction | Novobiocin |
|---|---|---|---|
| *S. aureus* | 0 | 46.53% | 91% |
| *P. aeruginosa* | 0 | 3.16 | 89% |

Results of insecticidal activity of n-hexane fraction of *A. triflora* showed significant potential for the control (100% mortality) (Table 2). However, unable to exhibit control potential against other four insect species. Contrarily, ethyl acetate fraction of *A. triflora* was ineffective against all studied insects. These results showed that promising insecticidal potential of n-hexane fraction can be due to specific phytochemical compounds found in the plant extract (Mostafa et al., 2016; Sayono et al., 2022).

Rozirwan et al., (2023) investigated insecticidal potential of mangrove leaves (*Avicennia marina* and *Excoecaria agallocha*). They found that methanol extract of from these leaves displayed insecticidal activity. GC-MS analysis extracts revealed the presence of diverse bioactive phytochemicals such as alcohol, lauric acid, myristic, linoleic, elaidate, stearate, endogenous, olead, phthalic ester, and siloxane (Rozirwan et al., 2023).

Alkaloids, flavonoids, lectins, phenolic acids, terpenoids and other bioactive compounds present in the plants can be attributed to the promising insecticidal activity of various plant-based extracts. Although extracts of different plants have shown promising results as natural insecticides, however, detailed research is required to extend the range of insect species, increase the efficacy and better understanding of insecticidal mechanisms of plant-based insecticides (Collares et al., 2023).

### 3.3. Antibacterial Activity

Two fractions (ethyl acetate and n-hexane) of *A. triflora* were examined to evaluate the antibacterial activity against five bacteria species by use of micro dilution method. Results showed that antibacterial activity of ethyl acetate fraction moderately inhibited (46.57%) *S. aureus* population (Table 3). However, n-hexane fraction showed no activity against any of the bacteria species evaluated in this study. Similar results have already reported and support the findings of present study (Mostafa et al., 2018). They concluded that extracts of *Cuminum cyminum, Punica granatum, Syzygium aromaticum, Thymus vulgaris* and *Zingiber officinales* showed antimicrobial activity against foodborne bacteria. Therefore, extracts of these and other plants can be used as potential natural alternative preservatives (Hossain et al., 2023; Islam et al., 2024; Mostafa et al., 2018; Sweet et al., 2024).

The antimicrobial potential of these and other plants are likely due to the presence of alkaloids, coumarins, flavonoids, glycosides phenolic acids, terpenoids and other bioactive compounds having promising antimicrobial activity (Álvarez-Martínez, et al., 2021; Asghar et al., 2022; Barbieri et al., 2017; Plabon et al., 2021). Bioactive compounds combat bacteria through diverse mechanisms, including cell membrane disruption, inhibition of cell wall formation, interfering various metabolic processes, inhibition of protein synthesis (AlSheikh et al., 2020; Chandra et al., 2020; Górniak et al., 2019; Sharma et al., 2021).

## 4. Conclusion

This study investigated the medicinal potential of *A. triflora*, an important member of the plant family Caprifoliaceae. The GC-MS analysis confirmed the presence of 36 phytochemicals which include several stigmasterols, fatty acids, vitamins, phenols, etc. The antibacterial activity showed that the ethyl acetate fraction of *A. triflora* extract showed potential against the *S. aureus* and limited activity against the *P. aeruginosa*, while n-hexane fraction lacked antibacterial activity to control any of the bacteria evaluated in this study. n-hexane fraction of *A. triflora* displayed strong insecticidal activity (100% mortality) against rice weevil (*S. oryzae*). However, there was no insecticidal activity observed against the other four insects. Ethyl acetate fraction of *A. triflora* extract lacked insecticidal potential against the evaluated five insect species. Observed 36 phytochemicals identified from different fractions extracted from *A. triflora* can have significant medicinal potential and this plant can be used in extensive research programs aimed at the development of plant-based medicines.

**List of Abbreviations:** GC-MS, Gas Chromatography-Mass Spectroscopy.

**Competing Interest Statement:** The authors have declared that they have no competing interests and there is no conflict of interest exists.

**Author's Contribution:** Sahifa and S. Ali designed the study. Sahifa and M. Bibi collected the samples. Sahifa wrote the initial draft of manuscript and reviewed it. All

the authors read and approved by final version of the manuscript..

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