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RESEARCH ARTICLE

Phytochemical composition and repellency activity of *Tephrosia vogelii* leaf extracts against *Aphis fabae* Scopoli (Hemiptera: Aphididae)

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ABSTRACT

Global efforts to control crop pests have traditionally depended on inorganic insecticides, which unfortunately had harmful effects on the ecosystem hence sustainability and the importance of maintaining ecological balance such use of plant extracts has gained momentum. This study aimed to determine the chemical makeup and repellency activity of *Tephrosia vogelii* leaf extracts prepared using methanol and dichloromethane solvents. In addition, a one-way experiment in a completely randomized design with 3 replications was used to evaluate the repellency activity of *Tephrosia vogelii* leaf extracts against *Aphis fabae* at different concentrations and conducted at 1 hrs intervals for 5 hrs. Phytochemical screening assays of *Tephrosia vogelii* leaf extracts indicated the presence of secondary metabolites such as flavonoids, anthraquinone, tannins, saponins, alkaloids and terpenoids. The LC-MS analysis identified 12 compounds. There is no clear evidence on the report of compounds such as 4,7-Dimethoxyisoflavone, Puerarin, 6,4-Dimethoxy-7-hydroxyiflavone, 3-hydroxy-7,8,4-trimethoxyflavone, curcumol, and Glycetein in *Tephrosia vogelii*. For all extracts of *Tephrosia vogelii* leaf, treatments revealed a significant repellency activity against black bean aphids ranging from 38.33% to 75 % after 5 hrs of exposure time ($P \leq 0.05$). The comparison of the repellent effects of dichloromethane and methanol leaf extracts of *Tephrosia vogelii* indicated that dichloromethane leaf extract at 8g/ml exhibited the highest repellent activity (65.33%) than methanol leaf extract (61.67%) at similar concentration. Its effectiveness is potentially attributed to the presence of bioactive compounds such as deguelin, rotenone, and quercetin. However, a detailed lab and field research using pure compounds is suggested.

Keywords: *Aphis fabae,* LC-MS analysis, Phytochemistry, Repellency activity, *Tephrosia vogelii*.

INTRODUCTION

Following scientific proof of the damage caused by inorganic pesticides, the awareness and use of plants with pesticide properties has steadily grown in less developed countries such as Tanzania (Mkindi et al., 2007; Malolo et al., 2024). Excessive use of inorganic pesticides accelerates environmental and health impacts (Mkindi et al., 2020). As a result, the use of plants with pesticide properties is gaining popularity, especially in developing countries (Mkindi et al., 2020). A complex mixture of secondary compounds in plant extracts has been reported to contribute synergistically to the effectiveness of active substances against pests and lower the resistivity development (Jacobs et al., 2015).

Tephrosia vogelii Hook F. is a pesticide plant belonging to the family Fabaceae (Leguminosae). The plant is commonly known as fish poisoning bean (or Vogel's tephrosia) (Mlozi et al., 2020). It is a soft woody herbaceous plant highly distributed in tropical and subtropical regions worldwide (Matovu & Olila, 2007; Yusuf et al., 2022). It grows well in areas receiving annual rainfall of around 850–2650 mm, a temperature range of 12.5 to 26.2 °C, and an altitude of up to 2100 meters above sea level (Kerebba et al., 2019). Tephrosia vogelii is a source of secondary metabolites including flavonoids, rotenoids, terpenoids, and sterols (Alhaithlou, 2023; Kayange et al., 2019). Tephrosia vogelii extracts are known to contain rotenoids that have pesticidal potential (Chemotypes 1). However, some lack rotenoids and they are referred to as chemotypes 2 (Kerreba et al., 2020).

Recent studies have discovered three distinct chemotypes of Tephrosia vogelii materials from East Africa (Kenya, Tanzania) and Malawi through phytochemical investigation (Mkindi et al., 2019). Stevenson et al. (2012) discovered chemotypes 1 and 2 of Tephrosia vogelii, and later, an additional Chemotype 3 was discovered by Mkindi et al. (2019). Chemotype 3 is the hybrid of chemotypes 1 and 2 (Mkindi et al., 2019). According to Zribi et al. (2014), chemotypes are defined as "subspecies of a plant that exhibit similar morphological features (form and structure) but tend to differ in types and quantities of chemical constituents. However, these variations in the phytocompounds would affect the pesticidal activities of these plants, putting restrictions on their use and delaying their adoption (Mkindi et al., 2019). A simple morphological feature for identifying *Tephrosia vogelii* and potentially distinguishing chemotypes would be flower colour, with typically white colour for chemotype 1, purple colour for chemotype 2, and a mixture of purple and white colour for chemotype 3 (Mkindi et al.,2019). However, a few plants with purple colour are chemotype I (Mkindi et al.,2019).

Many researchers have attempted to verify the utilization of Tephrosia vogelii as a natural pesticide under laboratory as well as field conditions (Mkindi et al., 2019; Mlozi et al., 2022). Rotenoids from Tephrosia vogelii Hook. F. (Leguminosae) including sarcolobines, deguelin, rotenone, and tephrosin are important as pest management and organic extracts for soil enrichment agents for resource-constrained small-scale farmers in Eastern and Southern Africa (Neme and Tole, 2019; Mkenda et al., 2015). Characterization and identification of new botanical extracts with pesticidal potential have been highlighted as critical steps in the development of environmentally safe insecticides (Sampson et al., 2005). In Tanzania, however, information on the chemical profile of naturally occurring Tephrosia vogelii plants remains limited. Additionally, due to variations in the chemistry (chemotypes) of Tephrosia vogelii, farmers may unwillingly utilize ineffectively plant materials, which could negatively accelerate the adoption and commercialization of plant extracts. In previous studies, chemotype 2 was reported to be ineffective against various crop pests due to the absence of compounds with pesticidal attributes such as rotenoids (Belmain et al., 2012). It is thus important to evaluate the phytochemical profiles of Tephrosia vogelii mostly occurring around farmer fields in Tanzania and to ascertain their pesticide potential. The objective of this study was to establish the chemical profile and the repellency properties of Tephrosia vogelii leaf extracts against aphis fabae found in Morogoro, Tanzania.

MATERIALS AND METHODS

Plant Materials collection

Tephrosia vogelii leaves were collected during the rainy season from several forest locations at Morogoro in January 2023. The *Tephrosia vogelii* plant from which the leaves were collected uniformly were characterized by not too old, tender, and green leaves, elliptic-oblong with white flowers; Seeds were oval shape, and the obliquely transverse and linear pod with slightly turgid shape. Collected leaves were air-dried under shade for four days before being ground into a powder of 200g. Before further usage, the powder was packed into an airtight container and stored in dry conditions under ambient temperature 25± 3°C and 65 ± 5°C RH.

Figure 1. Closer view of *Tephrosia vogelii* plant aerial parts.

Extraction

Extraction of phytochemicals was conducted according to Mlozi et al., (2022) with minor modifications. A powder sample of 200g was measured and successively soaked for 48 hours in a separate conical flask containing the respective amounts of dichloromethane and methanol, respectively. To prevent the thermal degradation of volatile chemicals, all solvents used were thoroughly evaporated using a rotary evaporator and low pressure at temperatures less than 40°C. For bioassay and phytochemical studies, *Tephrosia vogelii* leaf extracts were then kept at 4ºC. The percentage yield of the plant extracts was obtained following the formula proposed by Mussa et al. (2024):

$$\text{Yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \times 100$$

Rearing of the Aphis fabae Scopoli and preparation of the bean seedlings/crop

Before the laboratory trial, Black bean Aphid (*Aphis fabae*Scopoli) were reared in cages to get a cohort of the same age. A total of 40 aphids were inoculated to common bean plants for multiplication. Common bean seeds were sowed in 20 plastic pots filled with sterilized black loam soil. Pots were stationed on top of benches in the screen house in a controlled environment with a temperature of 26 ± 2°C and relative humidity of 65 ± 5%. Standard management practices were applied to the common bean plant. After multiplication aphids were collected for laboratory studies.

Screening for phytochemicals

Screening for phytochemicals was conducted according to Mlozi et al. (2022), Laizera & Mbwambo, (2022) &Alhaithloui et al. (2023) with slight modifications. *Tephrosia vogelii* leaf extracts were subjected to methanol and dichloromethane solvents, where different secondary metabolites were examined such as tannins, steroids, resins, terpenoids, alkaloids, saponins, and anthraquinone. Each chemical test was performed in duplicate to ensure consistency of results and reproducibility of the experiments.

Test for tannins

Tannins determination was conducted according to Mlozi et al. (2022). 1ml of distilled water (H_2O) and 0.5 gm of the extracted sample was mixed and filtered, the filtrate was mixed with a few drops of 5% ferric chloride. The development of blue-black precipitates confirmed the presence of tannins.

Test for steroids

Steroid determination was conducted according to Mlozi et al. (2022). Extract samples of 0.5g were dissolved in 2 ml of chloroform. Then, 1 ml of acetic anhydride was added to the mixture. Two drops of concentrated sulfuric acid were then gently added to the extract in the test tube. A reddish-brown colouration indicated the presence of a steroidal ring.

Test for terpenes

Terpenes determination was conducted according to Mlozi et al. (2022). Extract samples of 0.5 g were dissolved in 2 mL of chloroform. Then, 1 ml of acetic anhydride was added to the mixture. Two drops of concentrated sulfuric acid were then gently added to the liquid in the test tube. Colour changes from violet to pink-red showed the presence of terpenoids.

Test for saponins

Saponins were determined according to Mlozi et al. (2022). Around 0.5 grams of the extract sample were added to 5 ml of distilled water (H2O) and thoroughly shaken. The mixture was then warmed slowly. Even after warming, persistent foaming confirmed the existence of saponins.

Test for flavonoids

Flavonoids were determined according to Mlozi et al. (2022). 15 ml of distilled water (H_2O) was mixed with 0.25 grams of extract sample, and the mixture was then filtered. 5 ml of the filtrate was then collected and added to a test tube. The filtrate was then mixed with 5 ml of 20% sodium hydroxide. The presence of flavonoids was confirmed by yellow colour formations when the reagent was applied to the filtrate.

Test for anthraquinones

Anthraquinone determination was conducted according to Laizera&Mbwambo et al. (2022). 50 milligrams of extract sample was heated with 1 ml of a 10% ferric chloride solution and 1 ml of concentrated hydrochloric acid (conc. HCL). The resultant leaf extract sample was then cooled and well-filtered, and the filtrate was thoroughly well-shaken with an equal amount of diethyl ether. Ether extracts with strong ammonia formed a pink-red colouration in the aqueous layer, indicating the presence of anthraquinone.

Test for resins

Resins determination was conducted according to Alhaithloui et al. (2023). The 1 ml of extracts sample were mixed with a few drops of acetic anhydride solution and then, 1 ml of conc. H_2SO_4 was added. Yellowish to orange colouration indicated the presence of resins.

LC-MS Analysis

The experiment was conducted at the General Chemistry Laboratory Authority (GCLA), the LC-MS facility was used to determine the chemical composition of the dichloromethane and methanol extracts from *Tephrosia vogelii* leaves. 5 gm of powdered leaf sample was extracted for Liquid Chromatography Mass Spectrometry analysis by dissolving it in 10 mL of 99.9% methanol/dichloromethane of HPLC grade. The mixtures were then vortexed for two minutes, followed by 24 hours of incubation to ensure that they were well mixed. The mixtures were sonicated (ultrasonic microwave) for 15 minutes and later centrifuged at 1400 rpm for another 30 minutes. Therefore, a mixture of extracts and the mobile phase with water/acetonitrile (1:1 v/v) was thoroughly mixed and shaken to ensure good solubility before being injected into liquid chromatography-mass spectrometry. The solvent was kept at -5ºC during the LC-MS process. ThermoFisher Scientific's U300 mass spectrometer connected to a Q-active type mass spectrometer for ionization and scanning in both positive and negative modes via an APCI source was used for the identification of analytes. The acquisition parameters that were used included 45 eV impact energy for electron transfer dissociation with an MS2 scan type. The separation time was between 1 and 2 minutes, and 0.11% formic acid in acetonitrile was used as the eluent. Thermo Scientific Accuser RP-MS column measuring 100 mm × 4.6 mm × 2.6 mm at the column temperature of approximately 35 °C and flow rate of 300 ml/min was employed, nitrogen gas was used as the collision gas, and samples were scanned by Fourier-Transform Mass Spectrometry (FTMS) in the mass to charge ratio ranges from 300–780 to monitor the anticipated molecular ion range expected in the *Tephrosia vogelii* sample; each sample was monitored for a thirty-minute duration.

Experiment design and repellency activity

The experiment was performed at the Institute of Pest Management at the Sokoine University of Agriculture in the Morogoro region, from April to May 2023. The repellency activity was assessed using a Randomized Controlled Design (RCD) with three replications, which was based on the area preference approach proposed by Obeng-Ofori et al. (1998). Leaf extracts of *Tephrosia vogelii* were applied at concentrations of 2%, 4%, 6%, and 8% to assess their repellency effect against *Aphis fabae* after five hours. Cypermethrin 10% EC was applied at a rate of 1.5 mL/L of water, serving as the positive control. To set up the experiment, Whatman No. 1 filter paper circles in half, and 1 ml of the respective *Tephrosia vogelii* leaf extracts was applied to one half and the other half as a negative control (Water only). The filter papers were allowed to air dry to ensure the extracts were fully absorbed. For comparison, Cypermethrin ®25EC was used as a positive control. Each complete filter paper disc was placed in a Petri dish, where 10 live black bean aphids were released at the center. The trials were conducted in duplicate, counting the number of aphids in both treated and untreated areas at one-hour intervals for five hours. These counts were used to calculate the percentage repellency of each extract, following a formula from Gitahi et al. (2021).

$$\text{Percentage repellency (\%)} = \frac{CZ-TZ}{CZ+TZ} \times 100$$

Where,

CZ-Insect number on control zone; TZ- Insect number on treated zone; The repellency index was computed as follows;

Index of repellency (IR) = $\frac{2T}{T+C}$

Where, T= Number of aphids in the treated area; C=Number of aphids in the untreated area of preference.

The index of repellency values lies between 0 to 2 (Hiruy and Getu, 2023), with IR=1 stands for similar repellency, IR>1 indicating a lower repellency, and IR<1 stands for higher repellency (Gitahi et al., 2021).

Data Analysis

Data on the repellency effect were subjected to one-way analysis of variance (ANOVA) using the GENSTAT 16th Edition Statistical software program to determine the degree of significance. Fisher's LSD test was employed to separate mean values at P<0.05 level of significance.

The identification of compounds was done by comparing the mass spectra of the identified compounds with pre-existing spectra in the NIST library.

RESULTS

The yield percent of organic leaf extracts of *Tephrosia vogelii*

The results of the yield percent of organic leaf extracts of *Tephrosia vogelii* were 3.15 % and 2.55 % with their corresponding mass of 6.3g and 5.0 g, respectively.

Classes of Secondary metabolites present in Tephrosia vogelii leaf extracts

Leaf extracts of *Tephrosia vogelii* were subjected to preliminary phytochemical screening assays, which identified the presence of different secondary metabolites which are flavonoids, anthraquinone, alkaloids, tannins, saponins, as well as terpenoids (Table 1). Steroids and resins were not detected. These phytochemicals don't seem to have a purpose in physical or biological processes, but they help moderate interactions between plants with their biotic environment (Fatma & Bahia, 2013). It has been demonstrated that several of these phytochemicals have various pesticidal attributes against a wide range of crop pests.

Table 1. Phytochemical composition of *Tephrosia vogelii* leaf extracts

Tested phytochemicals	Methanol extracts	Dichloromethane extracts
Tannins	(+)	(+)
Steroids	(-)	(-)
Resins	(-)	(-)
Anthraquinone	(+)	(+)
Terpenes	(+)	(+)
Flavonoids	(+)	(+)
Saponins	(+)	(+)
Alkaloids	(+)	(+)

(+)-Present, (-)-Absent.

Compounds identified from Tephrosia vogelii leaf extracts

By comparing data of the liquid chromatography-mass spectrometry (LC-MS) with accessible NIST databases, the techniques were very useful in identifying the phytochemical components from methanol and dichloromethane leaf extracts of *Tephrosia vogelii.* As a result, twelve phytochemical compounds were discovered from the methanol and dichloromethane leaf extracts of *Tephrosia vogelii* using the LC-MS analysis of its molecular weights (m/z). The LC-MS analysis identified phytochemical compounds such as Puerarin, 4,7-Dimethoxyisoflavone, Curcumol, Aloin, Glycetein, Deguelin, Rotenone, Quercetin, isoquercetin, 3,4-dimethoxy-3-hydroxy-6-methylflavone, 6,4-Dimethoxy-7-hydroxyiflavone and 3-hydroxy-7,8,4-trimethoxyflavane (Table 2 & 3). Six compounds were identified from both dichloromethane and methanol leaf extracts (Table 3). LC-MS revealed that methanol and dichloromethane leaf extracts contained 10 and 8 phytochemical compounds, respectively. Out of the identified compounds, 4 from methanol leaf extracts and 4 from dichloromethane have been reported to possess different pesticidal properties essential for pest control. LC-MS analysis confirmed the presence of chemotype 1 in the analyzed sample due to the presence of rotenoids such as deguelin (Figure 2 & Figure 3).

Table 2. Compounds identified in methanol leaf extracts of *Tephrosia vogelii.*

Compound	RT	m/z	Formula	Compound name	Probability	Class
1	10.84	394.14	$C_{23}H_{22}O_6$	Deguelin	95.15	Isoflavonoids
2	10.28	284.07	$C_{16}H_{12}O_5$	Glycetein	80.8	Isoflavonoids
3	11.01	298.08	$C_{17}H_{14}O_5$	6,4-Dimethoxy-7-hydroxyiflavone	87.06	Isoflavonoids
4	12.48	464.1	$C_{21}H_{20}O_{12}$	Isoquercitin	82.25	Flavonoids
5	15.74	302.04	$C_{15}H_{10}O_7$	Quercetin	78.74	Flavonoids
6	9.852	312.1	$C_{18}H_{16}O_5$	3,4-dimethoxy-3-hydroxy-6-methylflavone	75.5	Isoflavonoids
7	9.48	416.11	$C_{21}H_{20}O_9$	Puerarin	96.8	Isoflavonoids
8	9.57	282.09	$C_{17}H_{14}O_4$	4,7-Dimethoxyisoflavone	79.97	Isoflavonoids
9	9.82	236.18	$C_{15}H_{24}O_2$	Cucurmol	81.8	Sesquiterpenoid
10	10.96	395.14	$C_{23}H_{22}O_6$	Rotenone	95.5	Isoflavonoids

Table 3. Compounds identified in dichloromethane leaf extracts of *Tephrosia vogelii.*

Compound	RT	m/z	Formula	Compound name	Probability	Class
1	10.14	394.14	$C_{23}H_{22}O_6$	Deguelin	98.95	Isoflavonoids
2	10.28	284.07	$C_{16}H_{12}O_5$	Glycetein	80.8	Isoflavonoids
3	11.01	298.08	$C_{17}H_{14}O_5$	6,4-Dimethoxy-7-hydroxyisoflavone	87.06	Isoflavonoids
4	12.48	464.1	$C_{21}H_{20}O_{12}$	Isoquercitin	82.25	Flavonoids
5	15.7	302.04	$C_{15}H_{10}O_7$	Quercetin	78.74	Flavonoids
6	10.96	395.14	$C_{23}H_{22}O_6$	Rotenone	95.5	Isoflavonoids
7	10.25	328.09	$C_{18}H_{16}O_6$	3-hydroxy-7,8,4-trimethoxyflavone	81.03	Flavones
8	9.6	418.13	$C_{21}H_{22}O_9$	Aloin	97.04	Anthraquinone

Table 4. Compounds identified in both dichloromethane and methanol leaf extracts of *Tephrosia vogelii.*

Compound	RT	m/z	Formula	Compound name	Probability	Class
1	10.14	394.14	$C_{23}H_{22}O_6$	Deguelin	98.95	Isoflavonoids
2	10.28	284.07	$C_{16}H_{12}O_5$	Glycetein	80.8	Isoflavonoids
3	11.01	298.08	$C_{17}H_{14}O_5$	6,4-Dimethoxy-7-hydroxyisoflavone	87.06	Isoflavonoids
4	12.48	464.1	$C_{21}H_{20}O_{12}$	Isoquercitin	82.25	Flavonoids
5	15.7	302.04	$C_{15}H_{10}O_7$	Quercetin	78.74	Flavonoids
6	10.96	395.14	$C_{23}H_{22}O_6$	Rotenone	95.5	Isoflavonoids

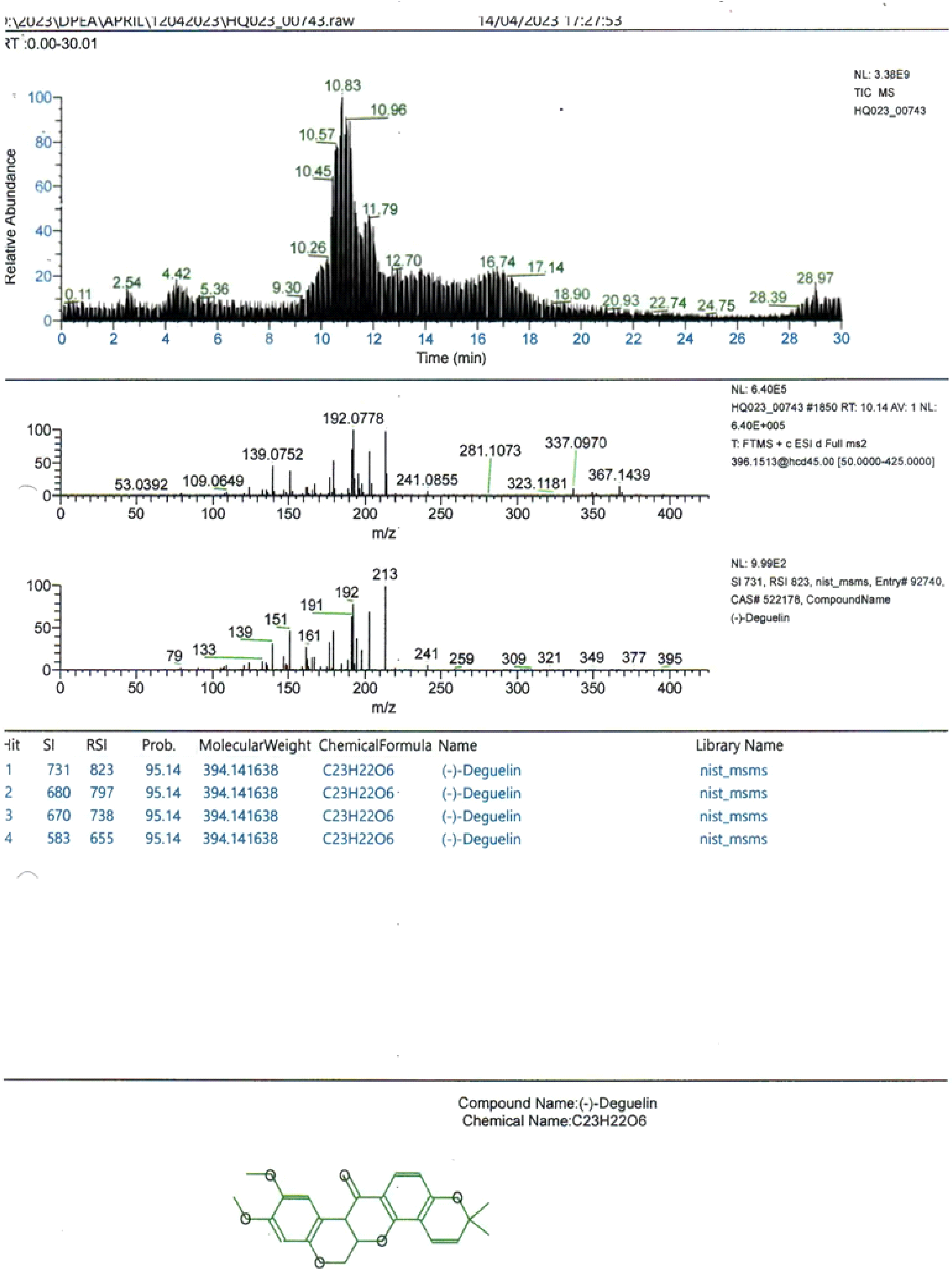
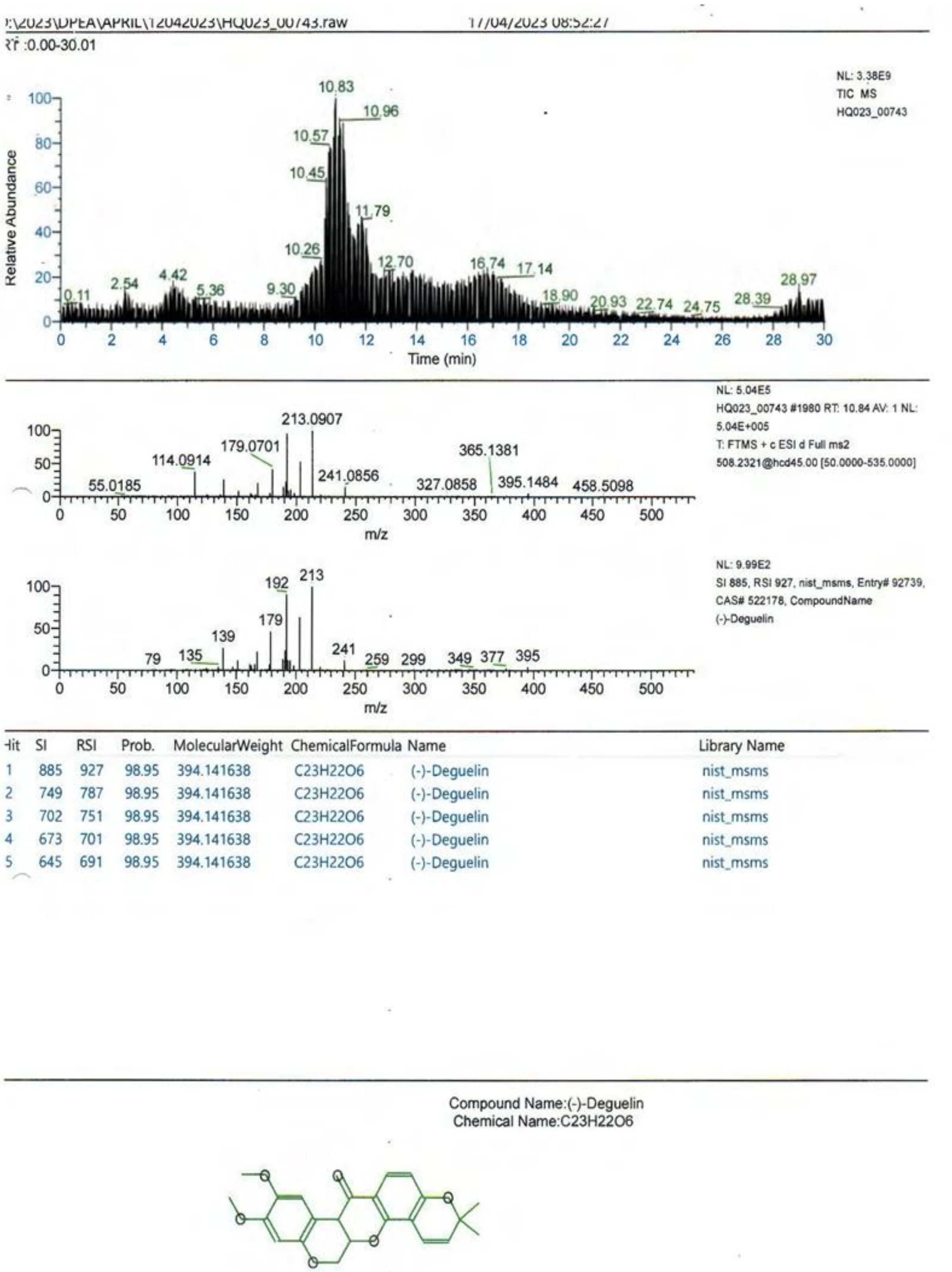


Figure 2. Representative chromatogram of methanol leaf extracts of *Tephrosia vogelii* for deguelin (chemotype 1) that show the relative intensity of retention time and mass to charge ratio (m/z).



Hit	SI	RSI	Prob.	MolecularWeight	ChemicalFormula	Name	Library Name
1	885	927	98.95	394.141638	C23H22O6	(-)-Deguelin	nist_msms
2	749	787	98.95	394.141638	C23H22O6	(-)-Deguelin	nist_msms
3	702	751	98.95	394.141638	C23H22O6	(-)-Deguelin	nist_msms
4	673	701	98.95	394.141638	C23H22O6	(-)-Deguelin	nist_msms
5	645	691	98.95	394.141638	C23H22O6	(-)-Deguelin	nist_msms

Figure 3. Representative chromatogram of dichloromethane leaf extracts of *Tephrosia vogelii* for deguelin (chemotype 1) that show the relative intensity of retention time and mass to charge ratio (m/z).

Changes of retention time with mass to charge ratio for compounds identified in methanol leaf extracts

Additionally, our investigation delved into the effect of mass-to-charge ratio on retention time for various chemical compounds. Figure 4 displays the appropriate data whereby; Quercetin (15.74 min) and Isoquercetin (12.48 min) had the highest retention times with respective mass to charge ratios of 302.04 and 464.01 respectively. Contrarily, Puerarin (9.48), 4,7-Dimethoxyisoflavone (9.57 min), and Curcumol (9.82 min) had the lowest retention times, with m/z ratios of 416.11, 282.09, and 236.18 respectively. The general trend is that the RT of chemical compounds is directly proportional to the mass to charge ratio. This observation implies that there is a higher possibility for the heavier molecules to elute from the column more slowly compared to the lighter molecules. It was also intriguing to find that among all the chemical compounds identified, Puerarin, with a high m/z of 416.11, displayed the lowest retention time (RT), suggesting that the high retention time value is due to a source other than the mass to charge ratio (Figure 4).

Changes of retention time with mass to charge ratio for compounds identified in dichloromethane leaf extracts

The longest retention time in Figure 5 was shown by Quercetin (15.74 min) and isoquercetin (12.48 min), which each had a mass-to-charge ratio of 302.04 and 464.01, respectively. On the other hand, the compounds with the lowest retention time were Aloin (9.59 min) with the respective mass-to-charge ratio of 416.11. The same as this, figure 5 proves that Aloin, with a high m/z of 418.11, had the shortest retention time of 9.6 min. This revealed that retention time was high due to variables other than mass-to-charge ratio.

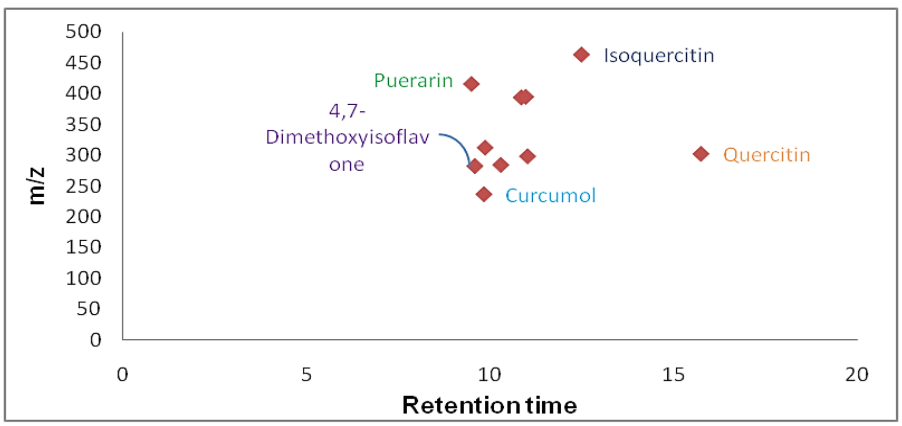


Figure 4. Scatter plot graph of precursor (m/z) against retention time (min) for compounds extracted by methanol leaf extracts.

Figure 5. Scatter plot graph of precursor (m/z) against retention time (min) for compound extracted by dichloromethane leaf extracts.

Repellent Activity of Methanol leaf extracts of Tephrosia vogelii against Aphis fabae.

The findings indicated a significant repellency effect of treatments on *Aphis fabae* population ($P \leq 0.05$), but no significant repellency effects were observed among *Tephrosia vogelii* leaf extracts after 2, 3, 4 and 5 hrs of spray ($p > 0.05$; Figure 6). The methanol leaf extract at concentration of 8% demonstrated the highest repellency, exceeding 50%, after one, two, three, four, and five hours of exposure to *Aphis fabae* (Figure 6). In contrast, the *Tephrosia vogelii* leaf extract at a concentration of 6% and 4 % exhibited the lowest repellency effect at only 38.3% and 40% respectively, at the final hour of the experimental period (Figure 6). Interestingly, the *Tephrosia vogelii* leaf extract concentrations of 2%, 4%, and 6% induced comparable repellent effects against *Aphis fabae* over the entire testing period ($p > 0.05$; Figure 6). Furthermore, after five hours of testing, the 8% leaf extract concentration showed repellent activity close to the positive control ($p > 0.05$).

Repellent Activity of dichloromethane leaf extracts of Tephrosia vogelii.

The findings indicated a significant effect of treatments on *Aphis fabae* population($P \leq 0.05$), but no significant repellency effects among *Tephrosia vogelii* leaf extracts after 2, 3,4, and 5 hrs of spray ($p>0.05$; Figure 7). Dichloromethane leaf extract at a concentration of 8% demonstrated the highest repellency percentage, exceeding 56%, after one, two, three, four, and five hours of exposure to *Aphis fabae*. Conversely, the *Tephrosia vogelii* leaf extract at concentrations of 6% and 4% showed the lowest repellency activity, recorded at 45% and 48.33%, respectively, at the final hour of the experiment (Figure 7). Throughout the test period, *Tephrosia vogelii* leaf extract concentrations of 2%, 4%, and 6% had similar repellent effects on *Aphis fabae* ($p > 0.05$). Furthermore, 8% leaf extract concentration showed repellent activity comparable to the positive control after 2 up to 5 hours of testing ($p > 0.05$; Figure 7).

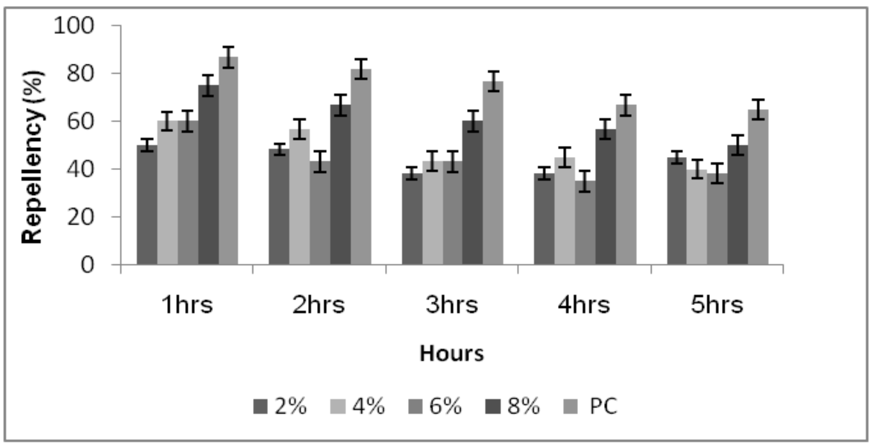


Figure 6. Percent repellency activityof methanol leaf extracts of *Tephrosia vogelii* against *Aphis fabae.*

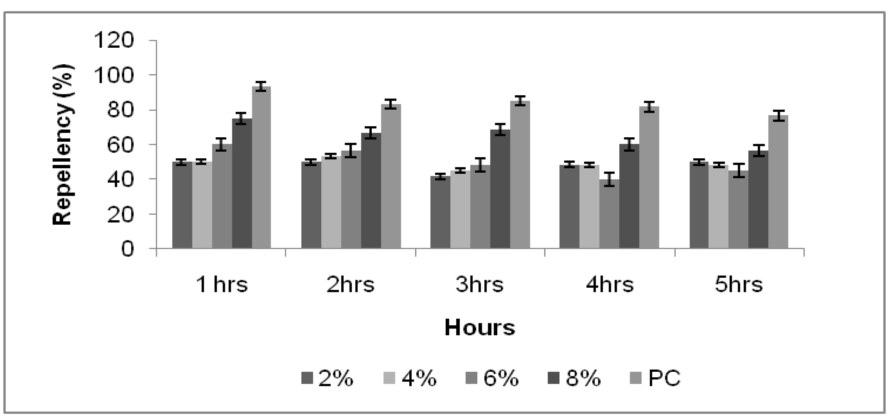


Figure 7. Percent repellency activity of dichloromethane leaf extracts of *Tephrosia vogelii* against *Aphis fabae.*

Comparison of the repellent effect of dichloromethane and methanol leaf extracts of Tephrosia vogelii.

The comparison of the repellent effects of dichloromethane (DCM) and methanol (ME) leaf extracts of *Tephrosia vogelii* indicated that dichloromethane leaf extract was significantly more effective than the methanol leaf extract. However, the dichloromethane leaf extract concentration of 8% showed a higher efficiency (65.33 %) compared to methanol leaf extract (61.67 %) at a similar concentration (Figure 8).

Repellency Index values of leaf extracts of Tephrosia vogelii against Aphis fabae.

The repellency index values indicated that the tested concentrations of methanol and dichloromethane leaf extracts at 8% showed greater repulsion to *Aphis fabae* compared to others (Table 4). Dichloromethane and Methanol leaf extracts of *Tephrosia vogelii* were compared on repellency index, and the results indicated that dichloromethane leaf extract (0.69) was more effective on repellent activity than the methanol leaf extract (0.77).

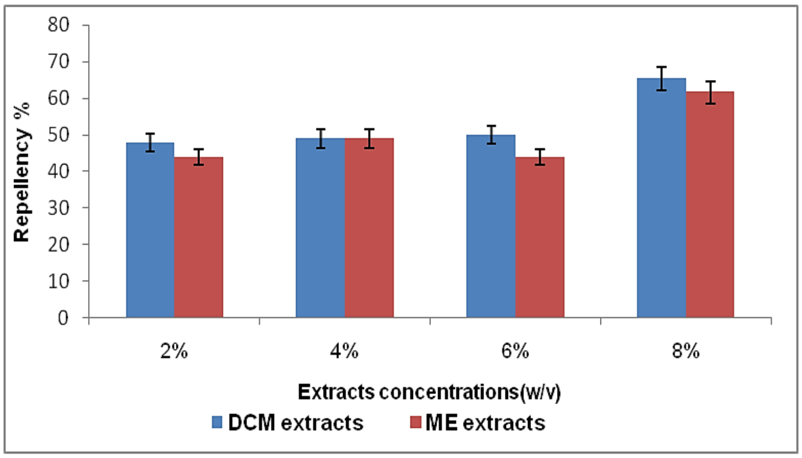


Figure 8. Comparison of repellency activities of the methanol and dichloromethane leaf extracts of *Tephrosia vogelii* against *Aphis fabae.*

Table 4. Repellency index values of two *Tephrosia vogelii* leaf extracts against *Aphis fabae*.

Conc. (g/100ml)	Methanol leaf extracts	Dichloromethane leaf extracts
2%	1.12	1.04
4%	1.02	1.02
6%	1.12	1
8%	0.77	0.69
Positive control	0.45	0.31

IR: <1 higher repellent; 1 neutral; >1 lower repellent

DISCUSSION

Phytochemical investigation

The secondary metabolites that the pesticidal plants such as *Tephrosia vogelii* produce determine how versatile they are against natural enemies. To maintain their survival, pesticidal plants can synthesize secondary metabolites of various functional groups that stimulate several chemical qualities (Mloziet al., 2022). Plant secondary metabolites have different functions, including helping plants to adjust to abiotic stressors like heat, drought, and UV radiation as well as coordinating interactions with mutualists and antagonists such as mycorrhizal fungi, rhizobium bacteria, herbivore predators and parasitoids, and neighboring plants (Kessler and Kalske, 2018). The study and characterization of putative secondary metabolites from pesticidal plants that may be exploited to create natural chemical products to treat human illnesses and agricultural pests has recently piqued the interest of researchers (Mlozi et al., 2022). However, recent studies have assessed many common pesticidal plant extracts, demonstrating that their application in smallholder farming can result in comparable yields to that when using commercial pesticides without causing serious environmental harm associated with synthetic compounds (Tembo et al., 2018; Phambala et al., 2020). Plants from the genus *Tephrosia* are renowned for being abundant in terpenoids, saponins, tannins, alkaloids, and other secondary metabolites (Tole and Neme, 2019).

Tephrosia vogelii leaf extracts were subjected to phytochemical screening tests, which identified various secondary metabolites such as tannins, alkaloids, anthraquinone, terpenoids, saponins, and flavonoids. The leaf extracts of *Tephrosia vogelii* lacked resins and steroids. Identified compounds in this study were reported from leaves of *Tephrosia vogelii* in other earlier studies (Dzenda et al., 2007; Isabella and Emma, 2017; Mlozi et al., 2022; Yadet and Tessema, 2020). Phytochemical screening tests confirmed the absence of resins and steroids suggesting that *Tephrosia vogelii* leaf extracts found particularly in Morogoro may be branded mainly by other compounds such as terpenes, tannins, saponins, flavonoids, anthraquinone as well as alkaloids. Tannins can resist and kill larvae; they affect the fecundity, development, and growth of many phytophagous insect pests (El-Aswad et al., 2023; Petchidurai et al., 2023; Djilali et al., 2021). Another secondary metabolite identified in this study was alkaloids. Although alkaloids mode of action varies, they primarily damage the nervous system's acetylcholine receptors or the membrane sodium channels, which results in death (Aly et al., 2022). Additionally, phytochemical screening tests confirmed the presence of terpenoids in the dichloromethane and methanol leaf extracts of *Tephrosia vogelii*. Terpenoids is thought to be helpful in agriculture activities especially in the creation of natural as well as synthetic pesticides for the proper management and control of various insect pests such as aphids (Laizera and Mbwambo, 2022; Kattel.,2023). The presence of saponins in *Tephrosia vogelii* leaf extracts can cause changes in the moulting process, feeding behavior, interactions with hormones that control growth, and even death at different growth phases of insect pests (De Geyter et al., 2007; De Geyter et al., 2011). Flavonoids play an important role in the protection of plants against plant-feeding pests such as aphids and herbivores (Harborne and Williams, 2000). Anthraquinone was also identified in *Tephrosia vogelii* leaf extracts. As a biopesticide, anthraquinone has been identified as a botanical insecticide and an insect repellent (DeLiberto and Werne., 2016). On the other point of view, the chemical nature of the phytocompounds analyzed from *Tephrosia vogelii* coincides with the pesticidal attributes reported in the previous studies (Zhang et al., 2022; Aly et al., 2022; Mlozi et al., 2022).

This current study also evaluated the presence and identity of biologically active compounds of dichloromethane and methanol leaf extracts of *Tephrosia vogelii* using LC-MS analysis. This study revealed the presence of 12 compounds in *Tephrosia vogelii*. LC-MS revealed that methanol and dichloromethane leaf extracts contained 10 and 8 compounds, respectively. Out of the identified compounds, 4 from methanol leaf extracts and 4 from dichloromethane have been reported to possess different pesticidal properties essential for pest control. Deguelin and rotenone found in this study were the same phytochemicals that were reported in *Tephrosia vogelii* leaves in previous studies (Stevenson et al., 2012; Antonio et al., 2019; Belmain et al., 2012; Mlozi et al., 2022). Our current findings were consistent with those results made public by Marston et al. (1984) who discovered molluscidal activities in *Tephrosia vogelii* as well as the presence of deguelin and Isoquercitin. Similarly, deguelin and rotenone were reported from the same species by Kalume et al. (2012). The findings of this study agree with the results of Stevenson et al. (2012) who reported the presence of isoquercitin in the leaf extracts of *Tephrosia vogelii.* These results corroborate with the findings of Dzenda et al. (2007) who revealed the presence of quercitin in the leaf extracts of *Tephrosia vogelii.* It was noted that 4,7-Dimethoxyisoflavone with chemical formula $C_{17}H_{14}O_5$ was reported from the seed of *Tephrosia purpurea* (Bhatnagar and Kapoor, 2000). Plants that share the same genus with *Tephrosia vogelii*suggest that they have the same ability to synthesize similar primary and secondary metabolites (Mlozi et al.,2022). There is no clear evidence on the report of the presence of Puerarin, Aloin, and Glycetein in the *Tephrosia vogelii* extracts though such phytocompounds have previously been reported from other Fabaceae plants such as *Scorpiurusmuricatus* L, and *Cassia fistula* (Barreira et al., 2017, Singh et al., 2012). The discovery of these compounds in *Tephrosia vogelii* demonstrates the biosynthetic pathways that plants in the same botanical lineage share, contributing to their similar properties in nature (Mhando et al., 2023). Other compounds such as 6,4-Dimethoxy-7-hydroxyisoflavone, curcumol and 3,4-dimethoxy-3-hydroxy-methylflavone were also identified in this study. There is no clear evidence that 6,4-dimethoxy-7-hydroxyisoflavone, curcumol, and 3,4-dimethoxy-

3-hydroxy-methylflavone have previously been reported in *Tephrosia vogelii* leaf extracts. This current study indicates the potential of these secondary metabolites' synthesis in plants from similar botanical lineages as well as adds to our understanding of *Tephrosia vogelii's* phytochemical profile.

From the pesticide value perspective, deguelin and rotenone have received praise in developing countries as promising natural pesticides (Kayange et al., 2019). LC-MS analysis confirmed that *Tephrosia vogelii* leaf extracts contain deguelin and rotenone which are toxic to various insect pests (Stevenson et al., 2012). These phytochemical compounds are known to be harmful to a wide range of crop pests. Mkenda et al. (2015) highlighted that the insecticidal actions are caused by the major phytochemicals' ability to work independently or in conjunction with minor constituents to increase their contact toxicity effects on insect pests. Deguelin has insecticidal activity against adult bruchids and aphids, as described by Zhang et al. (2022). Rotenone has been used to manage leaf-feeding insects, which include hemipteran species such as aphids and whiteflies, lepidopteron species such as caterpillars, and animal-related mites, spiders, beetles, carpenter ants, fleas, and lice (Zhang et al., 2022). According to Zhang et al. (2020), rotenone works by damaging electron transport at the mitochondrial level. This prevents phosphorylation of ADP to ATP which suppresses insect metabolism. Mpumi et al. (2016) denoted that rotenone is easily transported through the trachea into the bloodstream of insect pests, this is because their respiratory systems are directly connected to environments; for instance; the trachea of insect pests are not covered but they are just open to facilitate contact with the environment easily. Quercitin has insecticidal and repellent properties against herbivorous pests such as black bean aphids (Goławska, 2014). Surprisingly, quercitin inhibits the development and reproduction of insect pests, especially aphids, but does not affect beneficial insects (Riddick, 2021). Curcumol belongs to the sesquiterpene group, previously this group is known for its ability to deter pests from feeding (Tak et al., 2016). There is no clear evidence that 4, 7-dimethoxyisoflavone, 3,4-dimethoxy-3-hydroxy-methylflavone, Puerarin, Isoquercitin, 6,4-Dimethoxy-7-hydroxyiflavone, Aloin and Glycetein have previously reported to have pesticidal activities. When these bioactive compounds reach the insect integument, they can cause fundamental physiological, metabolic, biochemical and behavioral changes in the pests they kill.

Additionally assessed was how the mass-to-charge ratio of various compounds affected the retention time. The typical theory is that when m/z increases, the retention time of chemical compounds rises (Mhando et al., 2023). According to this theory, there is a higher possibility for the heavier molecules to elute from the column more slowly compare to the lighter molecules. From this study, the retention time was high due to factors such as stationary phase properties, mobile phase composition (such as polarity and solvent strength) (Mhando et al., 2023), as well as temperature but not mass-to-charge ratio. The current findings resemble the results of Mhando et al. (2023). An in-depth understanding of these factors is very important to optimize chromatographic conditions, hence improving accurate separation and reliable identification of diverse *Tephrosia vogelii* compounds in analytical applications. The findings of this study confirmed the hypothesis that *Tephrosia vogelii* leaf extracts contain potent phytochemicals. These results highlight the diverse and strong pesticidal properties present in the compounds produced by *Tephrosia vogelii,* highlighting their potential as efficient and environmentally responsible substitutes for conventional pest management techniques.

Repellency activity

The study also sought to evaluate the repellent properties of *Tephrosia vogelii* leaf extracts on *Aphis fabae*. All tested leaf extracts of *Tephrosia vogelii* exhibited potent repellent activity against *Aphis fabae* ($P \leq 0.05$). Over a 5-hour testing period, these extracts effectively repelled *Aphis fabae*, with most aphids avoiding the treated zones and remaining on the untreated zones of the Petri dishes. *Tephrosia vogelii* leaf extracts induced insect repellency ranging from 38.33 % to 75 % within the 5-hour experimental period (Figures, 6 & 7). These significant insect-repellent results align with the result findings of Benhizia et al. (2023) who reported that *Salvia microphylla* extracts could repel up to 62.59% of aphids. Increased *Tephrosia vogelii* leaf extract concentration led to a corresponding rise in the repellency activity against *Aphis fabae*, likely due to an increase in bioactive components with higher concentrations and vice versa. Gitahi et al. (2021) discovered similar results, demonstrating botanical extracts under laboratory circumstances become more repellent with the increase of the extract concentration. The observed escalation in aphid repellency with increasing extract concentration indicates the presence of powerful bioactive compounds such as deguelin, quercetin, and rotenone in the leaf extracts of *Tephrosia vogelii* that can be used to control various insect pests. Furthermore, the repellent activities were more pronounced for dichloromethane (DCM) leaf extracts compared to methanol (MEOH) leaf extracts, possibly indicating variations in the distribution of chemical constituents with pesticidal properties within *Tephrosia vogelii* leaf extracts. This variation in repellent activity underscores the crucial importance of understanding chemical variations influenced by factors such as plant parts, plant genotype, climatic conditions, harvesting time, region, and plant nutritional status, as documented in previous studies (Mkindi et al., 2019; Dhif et al., 2016). These results revealed that *Tephrosia vogelii* leaf extracts had a repellency effect against the *Aphis fabae* based on the repellency index scale of *Aphis fabae,* especially at 8g/ml composition.

CONCLUSION

Although *Tephrosia vogelii* leaf extracts display twelve different chemical compounds, as this study has demonstrated, it is necessary to recognize that some chemical constituents may be present that were below the instrumentation's detection threshold. Phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, terpenoids, anthraquinones, and saponins. LC-MS revealed that methanol and dichloromethane leaf extracts contained 10 and 8 phytochemicals, respectively. Out of the identified chemicals, 4 from methanol leaf extracts and 4 from dichloromethane have been reported to possess different bioactive attributes essential for pest control. The findings presented here provide a preliminary understanding of the phytochemical composition of *Tephrosia vogelii* obtained in Morogoro. The concentration-dependent repellency response, which suggests a dose-dependent effect was significantly lethal to *Aphis fabae*, indicates the presence of powerful bioactive compounds in the leaf extracts of *Tephrosia vogelii* that can be used to control various insect pests. The study's efforts were limited due to a lack of contextual information concerning the growth season as well as the age of the *Tephrosia vogelii* plants during harvest, both of which have the potential to exert influence over the chemical composition of the collected sample. Future research may include a methodical and controlled cultivation in which *Tephrosia vogelii* samples from the controlled cultivation and confiscated sample would be compared, with analysis considering the growing season and the age of *Tephrosia vogelii*during harvesting time. A detail lab and field research against aphids using pure compounds is suggested especially for compounds like 4,7-dimethoxy isoflavone,3,4-dimethoxy-3-hydroxy-methylflavone, Puerarin, Isoquercitin, 6,4-Dimethoxy-7-hydroxyiflavone, Aloin, and Glycetein*.*

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Not applicable

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AUTHORS CONTRIBUTION STATEMENT

DSN performed the conception or design of the work, data collection, data analysis, and interpretation and was a major contributor to the drafting of the manuscript. Furthermore, DSN also played a pivotal role in revising the work critically for important intellectual content, ensuring that the final document met the highest standards of quality and relevance in the field. PJL and ROM performed a critical revision of the article, providing critical comments concerning the discussion of results, conclusions, and recommendations.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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