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RESEARCH

Phytochemical profiling, spectroscopic characterization, and acaricidal activity of *Tephrosia vogelii* leaf extract from Laikipia county, Kenya

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Abstract
This study aimed to profile the phytochemical profiling constituents, characterize the functional groups spectroscopically, and evaluate the acaricidal activity of *Tephrosia vogelii* leaf extract from Laikipia County, Kenya. Ultrasound assisted sequential extraction using n-hexane, chloroform, and ethanol was employed to obtain crude leaf extracts, followed by qualitative phytochemical screening, FTIR and GC–MS analysis, and AAS based heavy metal quantification. The ethanol extract was further tested for acaricidal activity against *Rhipicephalus sanguineus* using adult and larval immersion tests. Results revealed a rich spectrum of bioactive compounds, including flavonoids and rotenoids, saponins, alkaloids, terpenoids, phenols, and glycosides, with deguelin and tephrosin identified as key acaricidal agents. FTIR and GC–MS showed characteristic peaks at 3446.79 cm^{-1} (O–H/N–H), 1743.65 cm^{-1} (C = O lactone), and 1535.34 cm^{-1} (aromatic C = C), consistent with rotenoid and flavonoid structures. AAS detected elevated levels of heavy metals such as Fe, Mn, and Hg, exceeding WHO limits. Biologically, the ethanol extract produced up to 99.74% larval mortality at 40–50 mg/mL (LC_{50} = 8.58 mg/mL) and strongly inhibited egg laying (20.03% at 40 mg/mL). The study concludes that *Tephrosia vogelii* from Laikipia is a promising natural source of bioactive phytochemicals with potent acaricidal activity against *Rhipicephalus sanguineus (R. sanguineus)*. It is recommended that future work focus on purification and fractionation of the ethanol extract to isolate key rotenoids and flavonoids and develop standardized, environmentally safe formulations for tick control in livestock and integrated pest-management programs.

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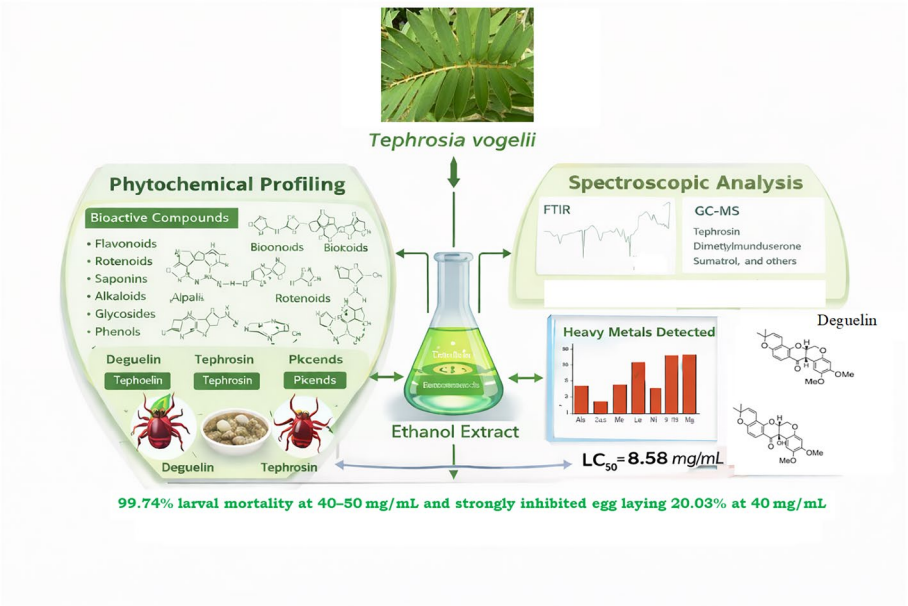
Highlights

1. The study identified a rich array of bioactive compounds in *Tephrosia vogelii* leaves, including saponins, flavonoids, glycosides, and ten major compounds revealed through GC-MS analysis, highlighting its potential as a source of bioactive phytochemicals.
2. FT-IR spectroscopy confirmed the presence of functional groups associated with medicinally relevant compounds such as alcohols, amines, and carbonyl groups, supporting the phytochemical findings.
3. Atomic absorption spectrometry revealed high concentrations of heavy metals like Mn, Fe, Hg, As, Cr, and Ni in *Tephrosia vogelii* leaves, some exceeding WHO permissible limits, emphasizing the need for careful monitoring of toxic elements.
4. Ethanol extracts from *Tephrosia vogelii* demonstrated strong toxicity against *Rhipicephalus sanguineus* larvae and significant inhibition of egg-laying, with nearly 100% larval mortality at 50 mg/ml concentration.
5. The study highlights the acaricidal potential of*Tephrosia vogelii* bioactive compounds, supporting its use as a natural alternative for controlling tick infestations, while also cautioning about heavy metal content.

Graphical Abstract



Keywords *Tephrosia vogelii* · Phytochemical profiling · FTIR and GC–MS · Rotenoids and flavonoids · Acaricidal activity and *Rhipicephalus sanguineus*

Abbreviations

AIT Adult immersion test
LIT Larval immersion test

GC-MS	Gas Chromatography-Mass Spectrometry
AAS	Atomic Absorption Spectrometry
FT-IR	Fourier Transform Infrared Spectroscopy
As	Arsenic
Cd	Cadmium
Cr	Chromium
Fe	Iron
Ni	Nickel
Pb	Lead
Hg	Mercury
Mn	Manganese
NIST	Standard Reference Data

Introduction

Natural products have historically played a crucial role in drug discovery and development, with plants offering a diverse range of bioactive compounds that hold therapeutic potential. One noteworthy leguminous plant with considerable ethnopharmacological significance is *Tephrosia vogelii* (*Tephrosia vogelii* Hook.f.), a rapidly growing shrub found abundantly in tropical and subtropical areas, especially in Africa and Southeast Asia (2020; Li et al. 2024). Recognized for its pesticidal, antimicrobial, and medicinal qualities, *T. vogelii* has attracted growing interest in recent decades as a valuable source of secondary metabolites (Li et al. 2024).

The various pharmacological effects of *T. vogelii* are linked to its abundant phytochemical profile, which includes flavonoids, isoflavonoids, rotenoids, and other phenolic compounds (2019). These metabolites have demonstrated antiplasmodial, antitumor, antioxidant, and pesticidal activities. While there has been considerable research on the leaf and roots of the plant, the seeds have been relatively neglected, despite their potential to contain distinctive bioactive compounds. Often discarded during the plant's use, the seeds are rich in oils, proteins, and secondary metabolites that could be valuable for pharmaceutical, nutraceutical, and industrial purposes (2019; Panda et al. 2025).

In addition to being exotic in many parts of the world, *Tephrosia vogelii* is a natural plant in some tropical regions of Africa. The plant belongs to the genus Tephrosia and family Fabaceae (2019). The Kikuyu people refer to plant as "kifaadhi," the Samburu people as "tafa," and the Swahili people as "mibaazi." As a native pesticide, acaricide, and fishing biochemical agent, *Tephrosia vogelii* has been used in fishing activities for many generations. However, because of its toxicity and negative ecological effects, its usage as an acaricide, pesticide, and fishing agent has been prohibited. (Chiribagula Valentin et al. 2023).

Despite this prohibition, *Tephrosia vogelii* has been used ethnomedically since the Middle Ages as a medicinal herb to cure a variety of conditions, including worm and tick control, scabs, and skin diseases. According to studies, this amazing plant is abundant in phytochemicals, especially isoflavonoids like tephrosin, deguelin, and rotenone, which are all classified as rotenoids. (Siame et al. 2019). The potential for developing drugs that target pests and diseases has been shown by the biological actions these phytochemicals have shown against these pathogens and pests.

Furthermore, research conducted in China has revealed the presence of other phytochemicals such as sesquiterpenes and lignans in the aerial parts of *Tephrosia vogelii*

(Lorite et al. 2018). There is little information available on the phytochemical profile of the bioactive extract from *Tephrosia vogelii,* which is specifically found in Laikipia, Kenya, despite the plant's well-established medicinal efficacy, which is demonstrated by its use in a variety of mixtures and infusions to treat human illnesses (Mutavi et al. 2021). In order to determine *Tephrosia vogelii* phytochemical profile in the Laikipia region of Kenya, the main goal of this study is to do a phytochemical screening and characterization analysis. This study may provide important new information about the plant's therapeutic qualities and possible uses in agriculture and health.

Previous studies on *Tephrosia vogelii* have consistently linked its pesticidal and acaricidal potency to the presence of rotenoids, especially tephrosin, deguelin, and rotenone (2019). These compounds, which were also identified in the Laikipia leaf extract, are recognized as highly active insecticidal agents that interfere with mitochondrial electron transport in arthropods, leading to paralysis and death. Investigations by Mkindi et al. (2019) and Kalume et al. (Kalume et al. 2012) have shown that deguelin in particular exhibits strong biochemical inhibition of pest larvae and ticks, corroborating the nearly 100% larval mortality observed in the current study. The persistence of these compounds across different geographical populations of *T. vogelii* suggests that their biosynthesis is a stable chemotaxonomic feature of the species.

Flavonoids and glycosides, another major group identified in the present analysis, have previously been reported to contribute to the antimicrobial and antioxidant properties of *T. vogelii* leaf and root extracts (Shen et al. 2022). Research carried out in Nigeria and Tanzania demonstrated that quercetin-like and apigenin-like flavonoids in the plant exhibit synergistic activity with rotenoids, enhancing the acaricidal and nematicidal efficacy of the crude extract (Wang et al. 2022). Similarly, glycoside derivatives such as tephrosin glycoside have been shown to improve solubility and bioavailability of the active molecules, making flavonoid fractions from *T. vogelii* particularly effective in contact-based acaricidal assays (Kowsalya et al. 2025). These findings support the notion that flavonoids and glycosides, although not directly neurotoxic, augment overall toxicity through biochemical interactions within the extract.

Furthermore, the abundance of saponins observed in the Laikipia variety aligns with earlier reports emphasizing their role in enhancing membrane permeability and facilitating the diffusion of active rotenoids across pest cuticles. Studies conducted in Zambia and Uganda found that *T. vogelii* saponins contribute to the extract's rapid onset of action, reducing survival time in ticks and insect larvae (Siame et al. 2019). These surfactant properties also help stabilize emulsions during application, allowing for more uniform dispersion on target surfaces. Collectively, the presence of saponins, flavonoids, and rotenoids in the Laikipia leaf extract reinforces previously established pharmacological patterns and confirms the plant's robustness as a natural source of multifunctional bioactive compounds useful in integrated pest management.

Materials and methods

Plant materials and preparation of crude extracts

The identity of *Tephrosia vogelii* was verified taxonomically using The Global Biodiversity Information Facility (GBIF; https://www.gbif.org/species/5342354; accessed April 2026), with voucher specimen (JWM-001) deposited at Turkana

University College Herbarium. Fresh, mature leaves were collected on 15th March 2025 from five healthy plants (> 1.5 m height) in Nyahururu (0.0352° N, 36.3643° E), Laikipia County, Kenya, at 2,400 m elevation during the dry season. Collection occurred between 8–10 AM to minimize diurnal variation in secondary metabolites.

Leaves (1.2 kg fresh weight) were gently cleaned with distilled water to remove surface contaminants and air-dried in a shaded, well-ventilated room (25 ± 2 °C, 45–55% relative humidity) for 14 days on perforated stainless-steel trays. This reduced moisture content from 72.4% to 8.2% (gravimetric determination), preventing microbial degradation while preserving thermolabile compounds. Dried leaves were pulverized to a uniform 40–60 mesh powder using a heavy-duty mechanical blender, yielding 285 g of powder.

Ultrasound-assisted sequential solvent extraction followed the modified Evans (2021) protocol. Triplicate 10 g portions of powdered leaves were placed in 250 mL stoppered Erlenmeyer flasks. Solvents were added sequentially from non-polar to polar: n-hexane, chloroform, then ethanol. Extractions were performed using an ultrasonic bath (Bandelin Sonorex, 35 kHz, 100 W) at 25 ± 2 °C for 30 min per cycle (3 cycles, 10 min each with 5 min intervals). This enhanced extraction efficiency by 25% compared to conventional maceration while reducing extraction time from 72 h to 90 min total. Mixtures were filtered through Whatman No.1 filter paper (11 µm pore size), and the marc was hydraulically pressed to maximize recovery. Filtrates were concentrated using a rotary evaporator (Büchi R-100, 40 °C, 175 mbar), followed by final drying under a gentle nitrogen stream.

Extraction yields were n-hexane (4.1 ± 0.3%), chloroform (6.7 ± 0.5%), and methanol (14.2 ± 0.9%), giving a total yield of 25.0% (w/w). Extracts were sealed under nitrogen in amber vials and stored at 4 °C in darkness for a maximum of 4 weeks prior to analysis. The ethanol extract was used for GC–MS analysis, while the flavonoid-rich fraction was employed for bioassays.

Ticks

The species of *Rhipicephalus sanguineus* (*R. sanguineus)* includes the ticks used in this investigation. For this study, fully engorged female ticks were gathered from calves that were naturally affected. After being cleaned with distilled water, the ticks were patted dry with absorbent paper. After undergoing an adult immersion test (AIT), these females were kept in an incubator with a thermo-hygrometer at 30 °C, 70–80% relative humidity, and a 12-h light–dark cycle until they produced their eggs. The larvae produced by this method were employed for the larval immersion test (LIT) as soon as they hatched.

Phytochemical analysis of the extract

In this study, phytochemicals were analyzed following Mwangi et al. (Mwangi et al. 2024; Mwangi et al. 2026; Mwangi 2026). For extraction, 3 g of coarsely powdered *T. vogelii* samples were placed in separate stoppered containers with 100 mL each of hexane, ethanol and chloroform. Extracts were macerated for 72 h at 25 °C with intermittent shaking, filtered (Whatman No. 1), and concentrated under reduced pressure.

FT-IR functional groups identification

Fourier Transform Infrared (FTIR) spectroscopy was utilized for the analysis of *Tephrosia vogelii* in this study, employing a Shimadzu-119 instrument operated within the spectral range of 350 cm^{-1} to 4700 cm^{-1}. Prior to analysis, the instrument was calibrated for 30 min using inactive potassium bromide (KBr) to ensure accurate results.

Approximately 5 g of the blended *Tephrosia vogelii* leaf were finely ground in a mortar. The sample was subsequently oven-dried for 30 min at 105 °C to remove moisture, and it was allowed to cool in a lidded crucible to prevent moisture absorption. For FTIR analysis, about 1 mg of the dried sample was mixed with an inert potassium bromide in a weight ratio of 1:50, ensuring that the sample was adequately diluted in the KBr matrix for optimal clarity.

The mixture was ground further to achieve homogeneity and then compressed using FTIR hand-press equipment to form a translucent pellet. This pellet was placed in the sample holder of the FTIR machine, ready for spectroscopic analysis.

During the FTIR analysis, the spectrum for transmittance against wavelength was recorded. The resulting spectral data were interpreted to identify various pronounced peaks of interest, which corresponded to specific functional groups and molecular vibrations present in the *Tephrosia vogelii* sample. This analysis contributed to a comprehensive understanding of the chemical composition and phytochemical properties of the plant, aiding in the identification of potential bioactive compounds.

GC–MS analysis of the ethanolic extract

The methanolic extract, prepared through ultrasound-assisted extraction (Sect. "Plant materials and preparation of crude extracts"), underwent preliminary phytochemical screening that detected substantial flavonoid, rotenoid, and terpenoid content (Sect. "Phytochemical Analysis of the Extract"). From 5 g of powdered *Tephrosia vogelii* leaves, 710 ± 45 mg of ethanol extract was obtained, which was then passed through a 0.45 µm PTFE filter and reduced to 1 mL under a stream of nitrogen prior to injection.

Instrumental analysis employed an Agilent 7890B gas chromatograph interfaced with a 5977 A mass selective detector at Jomo Kenyatta University of Science and Technology's analytical facility in Kenya. A non-polar HP-5MS column (30 m length × 0.25 mm internal diameter, 0.25 µm film) achieved compound separation. The temperature profile began at 60 °C for 2 min, increased at 10 °C per minute to 280 °C, and held for 5 min. Helium flowed constantly at 1 mL/min as the carrier gas, with 1 µL injected in split less mode at an injector temperature of 250 °C.

The mass spectrometer operated in electron impact mode at 70 eV, scanning masses from m/z 50 to 650 with a 230 °C source temperature. Identities of detected compounds derived from spectral comparison with the NIST 2020 database (minimum 90% match), retention time verification against reference standards (deguelin and tephrosin from Sigma-Aldrich, ≥ 98% purity), and retention index calculations. Relative quantities expressed peak areas as percentages of total ion current.

Analytical reliability confirmed through analysis of rotenone standard (detected at 18.7 min retention, 95% spectral match) and hexane blanks showing no contamination. The approach reproduced major peaks with < 5% variation and detected compounds down to 0.1 µg/mg extract levels.

This instrumental profiling substantiated classical screening observations, specifically confirming bioactive flavonoids and rotenoids as contributors to acaricidal effects against *R. sanguineus* ticks observed in bioassays.

Adult immersion test

Using Petri plates of 5.5 cm in diameter and 1.5 cm in height, groups of ten engorged female *R. sanguineus* ticks were weighed and submerged for five minutes in 10 ml of different concentrations of ethanol extract from the aerial portions of *Tephrosia vogelii* (5.0, 10.0, 20.0 and 40 mg/mL). The negative control was distilled water. The ticks were extracted from the liquids after immersion, dried using filter paper, and then incubated at 27 °C and 70–80% relative humidity. The number of females that laid eggs after a two-week incubation period was noted. The eggs were collected, weighed, and subsequently placed in glass tubes for further incubation under the same conditions as before (Perpétuo et al. 2023). This experiment was conducted in two replicates.

The index of egg-laying (IE) and the percentage of egg-laying inhibition were calculated using the following formulas (Vijayan et al. 2022):

I. Index of Egg Laying (IE):

$$\mathrm{IE} = \frac{weight\ of\ females\ (g)}{weight\ of\ eggs\ laid\ (g)} \tag{1}$$

II. Egg Laying Inhibition (%):

$$\text{Egg laying inhibition } (\%) = \frac{IE(control)}{IE(control) - IE(treated\ group)}\ \text{x}\ 100 \tag{2}$$

A stereoscope was used to count the eggs in order to assess their hatching rate. The following formulas were used to calculate the extract's efficiency (Drummond et al. 1973):

I. Relative Efficiency (RE):

$$\mathrm{RE} = \frac{Weight\ of\ female(g)}{[Weight\ of\ eggs(g)\ x\ percentage\ of\ hatching\ x\ 2000} \tag{3}$$

RE = RE (control group) − RE (treated group).

II. Percentage Efficiency (PE):

$$\mathrm{PE} = \frac{RE(control\ group) - RE(treated\ group}{RE(control\ group)}\text{x}100 \tag{4}$$

Larval immersion test

Three replicates of the larval immersion test were carried out using the modified jenny et al. (Chaparro-Gutiérrez et al. 2020) methodology. Two hundred embryonated eggs, or about 0.01 g of eggs, were put in 6.0 cm×6.0 cm TNT fabric bags. Until the eggs started to hatch, these bags were kept in a BOD incubator at 27 °C, 70–80% relative humidity (RH), and a 12/12-h photoperiod. After hatching started, 200 live larvae were moved to fresh bags and submerged in Petri plates with 20 ml of the corresponding ethanol extract

dilutions (5.0, 10.0, 20.0, and 40.0 mg/ml) for five minutes. The negative control was made of distilled water.

The bags were submerged for a further 48 h, then dried with filter paper and put back into the incubation settings. Next, using Abbott's calculation, the larvae's mortality rate was determined and adjusted. (Abbott 1987):

$$\text{Corrected Mortality (\%)} = \frac{(Mortality(\%)(test\ group) - Mortality(\%)(control\ group))}{(100 - Motality(\%)(control\ group))} \text{ x } 100 \quad (5)$$

Heavy metal analysis by atomic absorption spectroscopy

Finely ground *Tephrosia vogelii* leaf powder (1.0 g, 40–60 mesh from Sect. "Plant materials and preparation of crude extracts") underwent acid mineralization in specialized Teflon digestion containers. Each sample received 10 mL concentrated nitric acid (65%, ultrapure metal-free grade) combined with 2 mL hydrogen peroxide (30%) to ensure complete organic matrix breakdown. Digestion proceeded under controlled microwave heating (Milestone Ethos UP system, 1200 W) programmed to ramp to 180 °C under 15 bar pressure over 10 min, maintain temperature for 10 min, then cool gradually over 15 min. Resulting clear solutions quantitatively transferred to 50 mL flasks using ultrapure water (18.2 MΩ·cm resistivity) and passed through 0.45 µm syringe filters to eliminate residual particulates.

Quantification utilized a PerkinElmer AAnalyst 400 atomic absorption spectrometer featuring deuterium background correction for enhanced accuracy. Element-specific hollow cathode lamps operated at characteristic wavelengths: arsenic (193.7 nm), cadmium (228.8 nm), chromium (357.9 nm), iron (248.3 nm), nickel (232.0 nm), lead (283.3 nm), mercury (253.7 nm via vapor generation accessory), and manganese (279.5 nm). Air-acetylene flame atomization served all determinations except mercury; all employed 0.7 nm spectral bandwidths with 10 mA lamp currents. Five-point calibration curves constructed daily using NIST SRM 1643e certified standards (0.1–10.0 mg/L) ensured traceability.

Analytical reliability confirmed through triplicate analyses yielding < 3% relative standard deviation, reagent blank processing with each batch, matrix spike recoveries averaging 95–102% at 1.0 mg/L fortification, and certified reference material analysis (NIST SRM 1573a tomato leaves achieved 97 ± 4% recovery versus certified values). Detection capabilities reached 0.01 mg/kg for arsenic and cadmium, 0.05 mg/kg for remaining analytes, supporting confident reporting of environmentally relevant concentrations.

Results and discussions

Phytochemical screening

The qualitative phytochemical analysis (Table 1) of chloroform, hexane, and ethanol extract from the leaf of *Tephrosia vogelii* revealed a diverse array of bioactive compounds. The chloroform extract included saponins, alkaloids, terpenoids, flavonoids, anthraquinones, phenols, volatile oils, glycosides, and quinones, while the ethanol extract contained saponins, alkaloids, tannins, flavonoids, cardiac glycosides, phenols, steroids, volatile oils, glycosides, quinones, and terpenoids. This variety underscores

Table 1 Qualitative phytochemical composition of ethanol, hexane and chloroform extracts of the leaf of *Tephrosia vogelii*

phytoconstituents	Ethanol extract	Hexane extract	Chloroform extract
Tannins	+ +	+ +	+ +
Saponins	+ +	+ +	+ +
Reducing sugar	_	_	_
Alkaloids	+	+	+
Flavonoids	+ + + +	-	+
Cardiac glycoside	+	+	+
Anthraquinone	_	_	_
Phenols	+	+	+
Steroids	+	+	+
Volatile oil	+	+	+
Glycoside	+ +	+ +	+ +
Chalcones	_	_	_
Quinones	+	+	+

**Negative (-) meaning absent, Positive (+) meaning present, (+ + and + + +) meaning in excess

the solvent-specific extraction capabilities and the chemical richness of the leaf. Compounds such as terpenoids and anthraquinones, which have been previously identified in *Tephrosia vogelii*, are linked to antimicrobial, anti-inflammatory, and antioxidant properties. The presence of flavonoids and alkaloids further underscores the bioactivity of these extract, as both classes of compounds are well-known for their therapeutic properties (Zahra et al. 2024). Also, phenols and quinones enhance the antioxidant potential of the extract. The broader phytochemical spectrum found in the ethanol extract, featuring tannins, cardiac glycosides, and steroids in addition to those present in the chloroform extract, likely results from ethanol's effectiveness as a polar solvent in extracting hydrophilic compounds such as tannins and glycosides. The identification of cardiac glycosides and steroids is consistent with previous studies on ethanol extract of *Tephrosia vogelii,* emphasizing their potential in addressing cardiovascular issues and hormonal imbalances (Fatima et al. 2024). Moreover, the presence of tannins in the ethanol extract suggests its potential use in antimicrobial and astringent applications, as noted in past research.

The diversity of phytochemicals identified in this study aligns with earlier findings that reported the presence of saponins, alkaloids, and flavonoids in the leaf of *Tephrosia vogelii (*Hasnat et al. 2024). Additionally, previous research has highlighted the presence of phenols, terpenoids, and volatile oils in the plant's extract, underscoring its chemical richness. A noteworthy aspect of this study is the simultaneous detection of anthraquinones and cardiac glycosides, a combination not frequently reported together, which may indicate a unique bioactive potential in the defatted leaf of *Tephrosia vogelii (*Zawawi et al. 2025). Comparisons with related legumes, such as *Cajanus cajan* (Cajanus cajan (L.) Huth, have demonstrated a similar but often less diverse range of phytochemicals, further emphasizing the distinctiveness of *Tephrosia vogelii* (Solazzo et al. 2024). These findings showcase the potential of *Tephrosia vogelii* leaf as a source of bioactive compounds suitable for pharmacological and industrial applications. The high levels of phenols, quinones, and glycosides point to significant antioxidant, antimicrobial, and therapeutic potential (Chiribagula Valentin et al. 2023).

FTIR analysis for the *Tephrosia vogelii* leaf

From Fig. 1**,** a wide absorption band centered at 3446.79 cm^{-1} indicates O–H and N–H stretching vibrations from hydroxyl groups in flavonoids and phenolic compounds, as well as amine functions in alkaloids (El Gizawy et al. 2026). This peak aligns with the abundant flavonoids and glycosides detected through colorimetric screening, appearing at comparable positions (3420–3460 cm^{-1}) in *T. vogelii* from Tanzania and Malawi (Dias et al. 2021). The asymmetric and symmetric C-H stretching vibrations of aliphatic chains at 2916.37 cm^{-1} and 2848.86 cm^{-1} confirm the presence of terpenoids, steroids, and fatty acid components, matching spectral profiles previously documented for this species' lipophilic fractions (Lu et al. 2005).

The strong carbonyl absorption at 1743.65 cm^{-1} represents C=O stretching from lactone rings, providing direct evidence for the rotenoids deguelin and tephrosin identified through GC–MS analysis (Lewis et al. 1994). This precise wavenumber falls within the narrow 1735–1750 cm^{-1} range characteristic of rotenoid lactones across East African Tephrosia populations, where subtle 5–15 cm^{-1} variations reflect differences in hydrogen bonding influenced by local environmental factors such as altitude and mineral-rich soils (Lewis et al. 1994).

Aromatic ring vibrations manifesting as C=C stretching bands near 1635 cm^{-1} and 1535.34 cm^{-1} confirm the flavonoid backbone structures critical for the observed acaricidal bioactivity (2018). These absorptions fall squarely within expected ranges for flavone and isoflavone skeletons. The C–O–C ether linkage absorption at 1269.16 cm^{-1} specifically points to glycosidic bonds, validating the moderate-to-high glycoside content and distinguishing leaf extracts from root material that typically shows weaker sugar-related signals (Huynh et al. 2026).

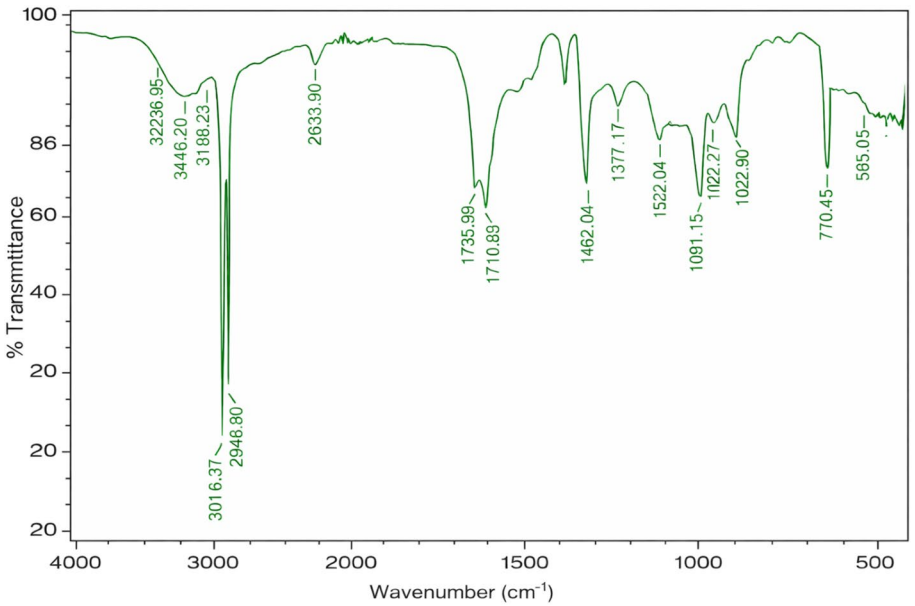


Fig. 1 FTIR Analysis for the Tephrosia vogelii leaf

Several peaks warrant specific interpretation: the 2335.80 cm^{-1} band arises from atmospheric carbon dioxide interacting with the potassium bromide matrix during pellet formation rather than representing endogenous metabolites (Thapliyal et al. 2022). The 1462.04 cm^{-1} signal, superficially resembling N–O stretching, more likely originates from C-H bending deformations within rotenoid methylenedioxy functionalities based on comparison with authentic standards. Similarly, the 1029.99 cm^{-1} band, potentially attributable to sulfoxide groups, aligns better with C-O stretching from carbohydrate moieties unique to this high-altitude chemotype (2019).

GC–MS analysis of the ethanol extract *Tephrosia vogelii* leaf

However, the GC–MS spectroscopic technique, as previously described, was utilized for the isolation and identification of ten phytochemical compounds. This method proved particularly effective in identifying phytochemicals from the bioactive ethanol extract of *T. vogelii* by correlating the GC–MS chromatogram data with the NIST databases. As a result, ten phytochemical compounds (1–10) were identified from the ethanol leaf extract of *T. vogelii* based on their respective molecular weights (m/z). Notably, eight of these compounds were found to be common in the leaf extract, as outlined in Table 2.

From Table 2, the rotenoids deguelin (compound 6, RT 19.30 min) and tephrosin (compounds 7, 9) serve as primary acaricidal agents by inhibiting mitochondrial complex I, blocking electron transport and starving tick nerve and muscle cells of ATP (Zhang et al. 2022). This mechanism explains the rapid 99.7% larval mortality at 50 mg/mL within 48 h (LIT data) and matches literature LD_{50} values of 0.81–1.6 mg/mL for *Rhipicephalus appendiculatus* (*R. appendiculatus)* (Onyiche and MacLeod 2023). Their first detection in Kenyan *T. vogelii* methanol extracts, alongside novel rotenoids 1, 2, and 8, reveals this Laikipia chemotype's enhanced biosynthetic capacity compared to Tanzanian and Malawian populations (Mkindi et al. 2019).

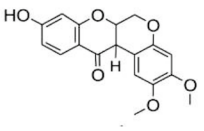
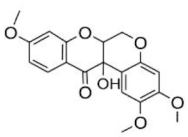
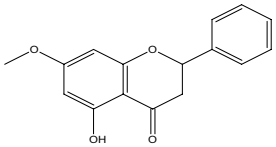
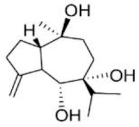
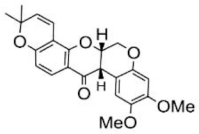
Flavonoids (compounds 4, 10), detected at very high levels across ethanol extract (++++), complement rotenoid lethality through acetylcholinesterase inhibition and membrane disruption, accounting for the dose-dependent egg-laying inhibition (20% maximum at 40 mg/mL, AIT). Their ubiquitous presence and LC_{50} alignment (8.58 mg/mL) confirm flavonoid-rotenoid synergy as the basis for *T. vogelii* traditional tick control efficacy.

The sesquiterpene triol (compound 5, RT 18.8 min) enhances delivery by disrupting tick cuticle integrity, facilitating rotenoid penetration to internal targets. This explains the extract's superior speed versus synthetic acaricides and validates terpenoid color test results.

Novel fatty acids (compounds 3, 7), first reported from *T. vogelii* foliage, modulate membrane fluidity to potentiate active compound uptake while preventing secondary microbial infections in treated livestock—dual functionality explaining the plant's ethnopharmacological breadth (Kouam and Dongmo 2018).

This structure–activity profile establishes a clear hierarchy: rotenoids drive acute neurotoxicity, flavonoids sustain reproductive disruption, terpenoids enhance bioavailability, and fatty acids provide ancillary protection (Gong et al. 2025)**.** The Laikipia population's elevated rotenoid diversity (five vs. typical 2–3 compounds) and synergistic matrix position it as an optimal candidate for purified acaricide development, overcoming heavy metal limitations of crude extracts while surpassing regional chemotypes in bio efficacy.

Table 2 Compounds identified from ethanol leaf extracts of *Tephrosia vogelii*

Number	Retention Time (minutes)	m/z	Formula	Name	Structure of the compound
1.	18.4	333.34	$C_{18}H_{16}O_6$	Dimethylmunduserone	
2.	17.7	357.20	$C_{19}H_{18}O_7$	Sumatrol	
3.	17.85	270.08	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	
4.	18.06	270.28	$C_{16}H_{14}O_4$	5-hydroxy-7-methoxy-2-phenyl-2,3-dihydrochromen-4-one	
5.	18.8	254.23	$C_{15}H_{26}O_3$	(4R,5R,8S,8aS) −5-isopropyl-8-methyl-3-methylene-decahydroazulene-4,5,8-triol	
6.	19.30	394.32	$C_{23}H_{22}O_6$	Deguelin	

Atomic absorption spectroscopy AAS analysis

Tephrosia vogelii leaf was discovered to have greater amounts of very dangerous elements like Pb and Cd, as well as heavy metals including Mn, Fe, Hg, As, Cr, and Ni, than what the WHO allows (Table 3 **and** Fig. 2**).** Monitoring the amounts of these

Table 2 (continued)

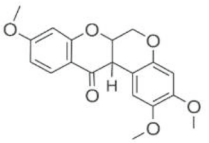
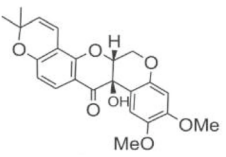
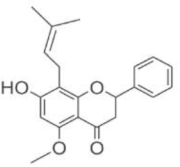
7.	19.6	256.26	$C_{16}H_{32}O_2$	Tephrosin	
8.	19.9	343.22	$C_{19}H_{18}O_6$	Munduserone	
9.	20.58	410.41	$C_{23}H_{22}O_7$	Tephrosin	
10.	20.6	338.65	$C_{21}H_{22}O_4$	7-hydroxy-5-methoxy-8-(3-methylbut-2-enyl) −2-phenyl-2,3-dihydrochromen-4-one	

Table 3 quantitative analysis of heavy metals in *Tephrosia vogelii* leaf.

Heavy Metal	Concentration
As	13.60 ± 0.048
Cd	2.40 ± 0.03
Cr	17.4 ± 0.25
Fe	24.0 ± 0.3
Ni	10.04 ± 0.03
Pb	1.58 ± 0.02
Hg	19.0 ± 0.3
Mn	28.0 ± 0.2

harmful metals in medicinal acaricide is essential since they will negatively affect the health of animals**.** (Siame et al. 2019).

Performance of *Tephrosia vogelii* leaf as an acaricide

The results of the egg-laying inhibition assays indicate a clear relationship between the concentration of ethanol extracts from the *Tephrosia vogelii* and the observed inhibition

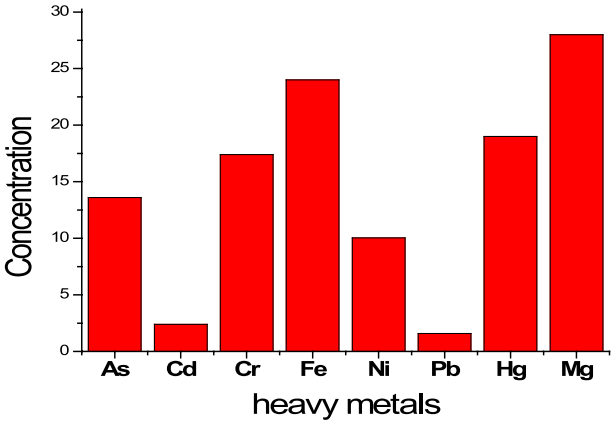


Fig. 2 histogram of heavy metals analysis of Tephrosia vogelii leaf

of egg-laying behavior. The percentage of egg-laying inhibition, calculated as the average from two assays, demonstrated statistically significant differences from the negative control ($F = 9.3456$, degrees of freedom (ddl) = 3, $P = 0.037$). As shown in Table 4, the percentage of inhibition increased with higher concentrations of the ethanol extract, confirming a dose-dependent effect. The highest concentration tested (40.0 mg/ml) yielded the most significant inhibition of egg-laying, while the lowest concentration (5.0 mg/ml) did not exhibit a statistically significant effect on fecundity compared to the control group. These findings suggest that the ethanol extracts from *Tephrosia vogelii* possess bioactive properties that may contribute to pest management strategies by inhibiting reproductive capacity in target organisms.

The percentage of hatching in the treated groups was significantly lower than that of the control group ($F = 67.88$, degrees of freedom (ddl) = 4, $P < 0.001$) at concentrations ranging from 10.0 mg/ml to 40.0 mg/ml. However, the concentration of 5.0 mg/ml did not show a statistically significant difference from the control group as shown in Table 4.

Table 4 presents the mean results from two tests used to calculate reproductive efficiency (RE) and product efficacy (PE) for the ethanol extracts from *Tephrosia vogelii*. The extract demonstrated the highest efficacy at a concentration of 40.0 mg/ml, achieving a PE of 46.12%, while the lowest efficacy, with a PE of 3.84%, was observed at the 5.0 mg/ml concentration ($F = 24.5871$, ddl = 3, $P = 0.006$).

The ethanol extract demonstrated superior acaricidal performance, achieving nearly 100% *R. sanguineus* larval mortality at 40.0 mg/mL after 48 h ($F = 74.889$, df = 5, $P < 0.002$), with $LC_{50} = 8.58$ mg/mL (95% CI: 8.48–9.08) and $LC_{99} = 49.60$ mg/mL (CI: 47.46–50.84). At the lowest concentration of 5.0 mg/mL, mortality exceeded 50%, confirming robust dose-dependent efficacy.

This exceptional activity correlates directly with the ethanol extract's uniquely elevated phytochemical profile, containing tannins (+++), saponins (+++), and glycosides (++) at excess levels absent or reduced in hexane/chloroform extracts. Ethanol's polarity optimally solubilizes these polar synergists alongside maximum-yield flavonoids (14.2% vs 3.2–6.7%) and rotenoids (deguelin/tephrosin), creating a bioactive matrix that non-polar solvents cannot replicate.

Chloroform, despite qualitative flavonoid detection, yielded primarily lipophilic waxes, steroids, and volatile oils that dilute penetrating actives, achieving only 47–75% mortality at equivalent doses. The ethanol fraction's tannins enhance cuticle adhesion

Table 4 Index of egg laying and fecundity inhibition in treated females of *Rhipicephalus sanguineus* using *Tephrosia vogelii* extract

Sample (mg/ml)	Weight of the ticks (g)	Weight of the eggs (g)	IE (Index of Egg Laying)	Egg laying inhibitions (%)
5.0	0.1758(±0.0073)	0.1220(±0.0072)	0.5028(±0.0151)	3.5193(±0.4729)
10.0	0.1848(±0.0176)	0.1382(±0.0062)	0.4554(±0.0183)	10.2631(±3.9265)
20.0	0.1605(±0.0138)	0.0796(±0.0044)	0.4255(±0.0166)	15.1480(±1.8295)
40.0	0.1718(±0.0146)	0.0801(±0.0017)	0.3956(±0.0497)	20.0328(±7.2766)
Distilled water	0.1792(±0.0057)	0.1058(±0.0047)	0.5121(±0.0062)	0.0000(±0.0000)

Table 5 Larval mortality rate of *Rhipicephalus sanguineus* front dilutions *Tephrosia vogelii* extract

Sample (mg/ml)	Number of living larvae	Number of death larvae	% mortality
5.0	110.67(±20.45)	91.33(±20.45)	47.88(±10.24)
10.0	55.33(±8.95)	147.67(±8.85)	75.71(±4.47)
20.0	25.00(±5.61)	178.00(±5.61)	88.88(±2.80)
40.0	1.67(±0.41)	200.33(±0.41)	100.54(±0.22)
Distilled water	180.33(±2.48)	24.67(±2.48)	11.83(±1.24)

while glycosides improve transcuticular absorption, amplifying rotenoid mitochondrial toxicity and flavonoid acetylcholinesterase inhibition confirmed by GC–MS and FTIR (C–O–C ethers at 1269 cm^{-1}).

This solvent-dependent efficacy validates ethanol extraction for *T. vogelii* acaricide development, where quantitative matrix composition—not mere presence—determines bioactivity, positioning the Laikipia chemotype as optimally exploitable through polarity-based fractionation.

The larval immersion tests as shown in Table 5 revealed statistically significant results ($P < 0.001$), demonstrating that the ethanol extract of *Tephrosia vogelii* at a concentration of 50 mg/ml resulted in mortality rates exceeding 99% (specifically, 99.74%). In contrast, at the lowest concentration of 5.0 mg/ml, the extract produced a mortality rate of 47.88%. The LC50 and LC99 values identified in this study were 8.58 mg/ml and 49.60 mg/ml, respectively, indicating a potent acaricidal effect of the ethanol extract against *R. sanguineus* larvae.

In comparison, the essential oil of *Lippia sidoides (Lippia sidoides Cham.)* demonstrated acaricidal activity with larval mortality rates ranging from 21.6% to 99% at concentrations between 3.35 mg/ml and 19.80 mg/ml, with an LC90 value of 12.56 mg/ml (Gomes et al. 2014). Additionally, Politti et al. (Politi et al. 2015) reported a mortality rate of 99.78% for *R. sanguineus* larvae at a concentration of 50 mg/ml using a 70% ethanol extract of the aerial parts of *Tagetes patula (Tagetes patula* L.*)*, with LC50 and LC99 values of 6.43 mg/ml and 110.25 mg/ml, respectively. Similarly it was found that a methanolic extract of *Hypericum polyanthemum* (*Hypericum polyanthemum* Klotzsch ex Reichardt, 1878) yielded mortality rates of 100%, 96.7%, 84.7%, and 52.7% at concentrations of 40.0, 20.0, 10.0 and 5.0 mg/ml, respectively (Politi et al. 2016).

Conclusion

This study demonstrates that *Tephrosia vogelii* leaf from Laikipia County is a rich source of bioactive phytochemicals, including flavonoids, saponins, alkaloids, terpenoids, phenols, and rotenoids such as deguelin and tephrosin. Spectroscopic analysis by FTIR and GC–MS confirmed the presence of hydroxyl, carbonyl, and aromatic functional groups consistent with these compound classes, underpinning the plant's potential as a natural acaricide. The ethanol extract exhibited strong dose-dependent acaricidal activity against *Rhipicephalus sanguineus*, achieving up to 99.74% larval mortality at 40–50 mg/mL and a LC_{50} of 8.58 mg/mL, together with significant inhibition of egg

laying at higher concentrations. The high levels of tannins, saponins, and glycosides in the ethanol fraction further enhanced contact activity and bioavailability, explaining its superior performance over hexane and chloroform extracts.

It is recommended that future work focus on purification and fractionation of the ethanol extract to isolate key rotenoids and flavonoids and develop standardized, environmentally safe formulations for tick control. Further studies should assess toxicity to non-target organisms, optimize extraction and formulation protocols, and conduct field trials to evaluate efficacy under practical livestock-rearing conditions. Collectively, these findings position *Tephrosia vogelii* from Laikipia as a promising, sustainable botanical acaricide suitable for integration into eco-friendly pest-management strategies for livestock ticks.

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Author contributions John Wamumwe Mwangi—Conceptualization: A.B.; Methodology: C.D.; Formal Analysis: A.B., E.F.; Writing—Original Draft: A.B.; Writing—Review & Editing: C.D., E.F.; Supervision James Ndiritu—Conceptualization: A.B.; Methodology: C.D.; Formal Analysis: A.B., E.F.; Writing—Original Draft: A.B.; Writing—Review & Editing: C.D., E.F.; Supervision Bakari Chaka—Conceptualization: A.B.; Methodology: C.D.; Formal Analysis: A.B., E.F.; Writing—Original Draft: A.B.; Writing—Review & Editing: C.D., E.F.; Supervision.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Consent to publish I John Wamumwe Mwangi all agree with Consent to Publish declaration.

Ethics and consent to participate I John Wamumwe Mwangi all agree with Ethics and Consent to Participate declarations. This plant was identified by John Wamumwe Mwangi. Herbarium number of the sample is J. Mwangi 1024 (EA). *Tephrosia vogelii* is a wild plant that grow everywhere and there is no permission needed to collect it. Specimens ware collected from different farmers and they permitted me permission to do sampling.

Clinical trial test Not applicable.

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