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Research Article

Phytochemical profiling of *Prosopis laevigata* and *Vachellia farnesiana* leaf extracts

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Abstract: Natural products are a rich source of compounds with diverse chemical structures and bioactivities. For instance, chemotherapeutics like paclitaxel, which belong to the alkaloid and terpene groups, have been isolated from plant sources. The present study aimed to obtain bioactive crude extract and its fractions from the leaves of *Prosopis laevigata* and *Vachellia farnesiana*, and to determine their total alkaloids and triterpenoid content. The crude extracts contained alkaloids at concentrations of 621.64 and 379.84 mg AE g^{-1}, and triterpenoids at concentrations of 335.60 and 364.72 mg UAE g^{-1}, with extraction yields of 2.64% and 2.58% concerning dry weight (DW), for *P. laevigata* and *V. farnesiana*, respectively. Both extracts were fractionated using a chromatographic column, yielding eight representative fractions for each extract. In *P. laevigata*, fraction 4 exhibited the highest alkaloid content (53.03 mg AE g^{-1}), while fraction 8 showed the highest triterpenoid content (69.93 mg UAE g^{-1}). In contrast, *V. farnesiana*´s fraction 5 contained the highest alkaloid content (86.04 mg AE g^{-1}), and fraction 2 had the highest triterpenoid content (88.37 mg UAE g^{-1}). HPLC-Q-TOF-MS/MS analysis of crude extracts revealed the presence of alkaloids from the cinchonine family, as well as 3-O-acetyl-16-α-hydroxytrametenolic acid, a cytotoxic triterpenoid. The identification of these bioactive compounds warrants further investigation into their cytotoxic activity against cancer cell lines.

1. INTRODUCTION

Many of the most effective and successful chemotherapeutic drugs used to treat cancer are of natural origin. Currently, researchers are developing semi-synthetic medications for cancer treatment, but many of these drugs come from plant sources (Mehta *et al*., 2022). Examples include vincristine and vinblastine, extracted from *Catharanthus roseus*, and paclitaxel (taxol®), extracted from the bark of the *Taxus* tree, a complex diterpene-alkaloid (Gusain *et al*., 2021). These drugs are used to treat various types of cancer (Sak, 2022). Alkaloids are cyclic compounds derived from amino acids, containing nitrogen in their structure (Cordell *et al*., 2001). They are widely distributed in plant species, particularly in those belonging to the Fabaceae, Papaveraceae, Menispermaceae, and Loganiaceae families (Lu *et al*., 2012). As one

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of the most active constituents of plants (Mondal *et al*., 2019), alkaloids exhibit a wide range of biological activities, making them promising anticancer agents. The main mechanisms of action include inhibiting cell proliferation, altering DNA replication, disrupting cell cycle progression, and inducing apoptosis (Nair & Van Staden, 2018). On the other hand, terpenes are molecules derived from isoprene, classified according to their carbon number. Terpenoids have been shown to be effective in preventing and treating various types of cancer. Certain terpenoids exhibit anti-cancer effects by inducing cell cycle arrest, inhibiting angiogenesis, or preventing metastasis (Kamran *et al*., 2022).

Mexico boasts a rich botanical diversity, yet only a small fraction of these plants has been studied. Research has focused on approximately 300 plants, belonging to 181 species, which have been investigated *in vitro* and *in vivo*. Notably, around 60% of these plants have exhibited cytotoxic activity against at least one cancer cell line. The Fabaceae, Asteraceae, Lamiaceae, and Convolvulaceae families have shown the highest number of species with *in vitro* bioactivity (Alonso-Castro *et al*., 2011). The genera *Prosopis* and *Vachellia*, both belonging to the Fabaceae family, comprise multipurpose species (Rodriguez-Franco & Maldonado-Aguirre, 1996). In Mexico, there are eleven species of *Prosopis*, commonly known as mesquite (Cedillo & Mayoral, 1997), and approximately seven species of *Vachellia*, previously known as *Acacia* (Kyalangalilwa *et al*., 2013), commonly referred to as huisache. Among the species, *Acacia farnesiana* (syn. *Vachellia farnesiana*) has been traditionally used to treat various ailments, including diarrhea, headache, and tuberculosis. Additionally, the ethanolic extract of *A. farnesiana* has been found to have *in vitro* activity against a chloroquine-resistant strain of *Plasmodium falciparum.* Similarly, *Prosopis laevigata* has been used to treat a range of conditions, including dysentery, abdominal pain, conjunctivitis, rashes, cough, fever, toothache, pharyngitis, and snoring. Studies have demonstrated antimicrobial properties of *P. laevigata* methanolic extracts, which have shown inhibitory effects against *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Escherichia coli,* and *Staphylococcus aureus* (Morales-Domínguez *et al*., 2019).

On the other hand, the genera *Prosopis* and *Vachellia* have also demonstrated potential in the preparation of extracts with cytotoxic activity against various cancer cell lines. For example, studies on *Prosopis juliflora* have revealed cytotoxic activity against human T-cell leukemia (Henciya *et al.,* 2017), as well as against MOLT-4 (Sathiya & Muthuchelian, 2011), MCF-7, HepG2, LS-174T (Elbehairi *et al*., 2020), B16-F10 (Cavalcante *et al*., 2021), A549 (Alshammari *et al*., 2024) cell lines, and a metastatic cancer murine model using B16-F10 cells (Choudhari *et al.,* 2025). Similarly, *Prosopis laevigata* has exhibited cytotoxic activity against SiHa, HeLa, Ca Ski, and C-33 A (Ibarra-Berumen *et al*., 2023) cell lines. *Prosopis cineraria* has shown activity against the K-562 (Sarkar *et al*., 2022) cell line. *Vachellia schaffneri* has demonstrated activity against colon, lung, and skin cancer cell lines (Manríquez-Torres *et al*., 2020), as well as HT-29, A-549, and UACC-62 (Torres-Valencia *et al*., 2015) cell lines, while *Vachellia farnesiana* has shown activity against HeLa (Domínguez-Colín *et al*., 2024) cell line.

To evaluate the cytotoxic activity of these compounds and to conduct subsequent preclinical studies, it is essential to separate them from the plant matrix using extraction techniques, solvent partitioning, and chromatography. These methods enable the obtention of fractions with reduced chemical complexity (Sarker *et al*., 2006). Therefore, the objective of this study was to obtain alkaloid- and triterpene-enriched extracts from *Prosopis laevigata* and *Vachellia farnesian*a, along with their corresponding fractions, to identify suitable fractions for subsequent cytotoxicity testing.

2. MATERIAL and METHODS

2.1. Plant Material

Leaves and specimens of *Prosopis laevigata* and *Vachellia farnesiana* were collected from San Juan del Río, Querétaro, México. The specimens were deposited in the herbarium of medicinal plants at the Mexican Institute of Social Security in Mexico City, where they were identified

and registered as follows: *Prosopis laevigata* (registry number 17002) and *Vachellia farnesiana* (registry number 16997).

2.2. Processing of Plant Material for Phytochemical Analysis

Fresh leaves of both plant species were weighed to determine their fresh weight (FW). The leaves were then dried using a freeze dryer (Labconco, Kansas City, MO, USA) to obtain their dry weight (DW). The dry biomass was subsequently crushed into a fine powder using a food processor and stored in an amber flask within a desiccator until further use. Moisture content (MC) was determined for each sample using the following equation:

$$MC=(FW-DW)/FW\times100 \quad (1)$$

Where MC is the moisture content (%), FW is the fresh weight, and DW is the dry weight.

2.3. Plant Extracts

The preparation of the extracts was based on the extraction protocol proposed by Wall *et al*. (1996), a modified version of the methodology described by Statz & Coon (1976). To prepare the extracts, 1 g of dry biomass was extracted with 30 mL of ethanol (Fermont, Mexico) under constant stirring at 100 rpm for 24 h. Subsequently, the mixture was subjected to ultrasonic bath extraction (SK2210HP, China) at 40°C and 53 kHz for 60 min, followed by filtration. To adjust the ethanol concentration to 90%, 2 mL of ethanol and 3.5 mL of distilled water were added to the sample. The sample was then extracted with hexane (Fermont, Mexico) in equal volumes (35.5 mL) using a separatory funnel, and the lower phase was recovered. The resulting extract was concentrated to dryness using a rotary evaporator (IKA RV 10, Germany). For further purification, 10 mL of chloroform (Fermont, Mexico) and 10 mL of distilled water were added to the sample, followed by partitioning with a 1% NaCl (J.T. Baker, USA) solution in equal volumes (20 mL). Finally, the chloroform extracts were dried using a rotary evaporator (IKA RV 10, Germany) and stored in amber flasks at 4°C. The resulting extracts were designated as crude extract from *P. laevigata* or *V. farnesiana.*

2.4. Extract Fractionation

The dry extract obtained from 5 g of biomass, as described in subsection 2.3, was resuspended in 2 mL of chloroform, and subsequently fractionated on a 400 x 20 mm glass chromatography column packed with 60 g of silica gel (Sigma-Aldrich, USA; 60-200 mesh) as the adsorbent. Fractionation was performed using a chloroform-methanol solvent system (Fermont, Mexico; 99:1 to 80:20). Five-milliliter fractions were collected and analyzed by thin-layer silica gel chromatography. The presence of alkaloids and terpenes was detected using Dragendorff's test and Liebermann-Burchard test, respectively. Additionally, the fractions were further analyzed under short- and long-wavelength ultraviolet light. Fractions with similar chromatographic profiles were combined and dried by lyophilization.

2.5. Thin-Layer Chromatography Analysis of The Extract and Its Fractions

The extract and its fractions were analyzed by thin-layer chromatography (TLC) using aluminium plates coated with silica gel 60 F254 (Merck-Millipore). A chloroform-methanol elution system (97:3) was employed for the separation. The developed plates were visualized using short- and long-wavelength ultraviolet light (254 and 365 nm, respectively) and further detected using Dragendorff's reagent and Liebermann-Burchard reagent. The retention factor (Rf) was calculated for each spot revealed.

2.6. Alkaloid Determination in Crude Extract and Its Fractions

The alkaloid content of the crude extracts (ethanol) was determined using the spectrophotometric method described by Shamsa *et al.* (2008). A standard curve ($R^2 = 0.995$) was constructed by dissolving 1 mg of atropine (Sigma-Aldrich, USA) in 10 mL of distilled water to create a stock solution. Aliquots of the stock solution were used to prepare different concentrations (50, 80, 100, 120, 150, and 200 µg mL^{-1}). To determine the alkaloid content, 0.1

mL (0.1 g mL^{-1}) of the crude extract or its fractions was placed in separate funnels. Then, 5 mL of bromocresol green (BCG, 0.1 mM) solution, prepared by heating 69.8 mg of BCG with 3 mL of 2 M NaOH and 5 mL of distilled water, was added to each funnel. Subsequently, 5 mL of Na_2HPO_4 buffer solution (0.5 M, pH 4.7) and 2 mL of chloroform were added. The organic phase was extracted, collected, and then re-extracted with an additional 3 mL of chloroform. The combined extracts were made up to a volume of 10 mL in a volumetric flask. The absorbance of each solution was measured at 470 nm using a spectrophotometer (Genesis 10S UV-vis, Thermo Scientific, USA). The results were expressed as atropine equivalents in milligrams per gram of dry extract (mg AE g^{-1}).

2.7. Triterpenoid Determination in Crude Extract and Its Fractions

The triterpenoid content of the crude extracts (ethanol) and their fractions was determined using the spectrophotometric method described by Numonov *et al*. (2020) with minor modifications. To construct the standard curve (R^2 = 0.998), 10 mg of ursolic acid (Sigma-Aldrich, USA) was dissolved in 50 mL of ethanol (Sigma-Aldrich, USA) to create a stock solution. Aliquots of the stock solution were used to prepare different concentrations (2, 4, 8, 12, and 16 µg mL^{-1}). For the analysis, 0.1 mL of the crude extract or its fractions was placed in a test tube with 0.4 mL of 5% vanillin (Sigma-Aldrich, USA)- glacial acetic acid (99.9 %, J.T. Baker, USA) solution and 1 mL of sulfuric acid (J.T. Baker, USA). The mixture was heated at 60 °C for 30 min, then cooled and diluted to 10 mL with glacial acetic acid. The absorbance of each solution was measured at 547 nm using a spectrophotometer (Genesis 10S UV-vis, Thermo Scientific, USA). The results were expressed as milligrams of ursolic acid equivalents per gram of dry extract (mg UAE g^{-1}).

2.8. HPLC-Q-TOF-MS Analysis of Crude Extracts

Crude extracts were dried under reduced pressure and reconstituted in HPLC-grade acetonitrile (JT Baker, USA) to achieve a concentration of 1 mg/mL. The solutions were then filtered through 0.2 µm, 15 mm membranes (Agilent, Germany) with a 33 µL injection volume. A 200 µL aliquot of each sample was analyzed using a high-resolution liquid chromatograph (Agilent, Germany) coupled with quadrupole time-of-flight mass spectrometry (Agilent, Germany). The chromatographic separation was performed on a C-18 column (5 µm, 250 mm × 4.6 mm i.d., Supelco Discovery), using a programmed gradient with a linear moving phase at a flow rate of 5mL/min. The mobile phase consisted of a binary gradient system with two solvents: A: 0.1% formic acid in water and B: acetonitrile. The gradient program was as follows: t = 0 min, 50% A, 50% B; t = 5 min, 55% A, 45% B; t = 10 min, 60% A, 40% B; t = 15 min, 65% A, 35% B; t = 17 min, 70% A, 30% B; t = 25 min, 50% A, 50% B.

Mass spectrometry analysis was performed using an Agilent Jet-Stream electrospray ionization (ESI) source operated in negative polarity mode. The ESI source parameters were as follows: TOF-MS acquisition mode, mass range: 50-950 *m/z*, drying gas: N2 at 180°C and 0.30 mL/min, nebulizer pressure: 40 psi, nozzle voltage: 1000 V, sheath gas: 300°C and 10 L/min, capillary voltage: 4000 V, skimmer voltage: 65 V, fragmentor voltage: 150 V, octapole RF: 750 V. Compound identification was achieved by comparing the obtained spectra with the MassBank 2023.11 spectra library. Molecular formula determination and exact mass calculation were performed using ChemCalc, with an error tolerance of less than 10 ppm compared to the calculated (exact) monoisotopic masses (Geng *et al*., 2023).

2.9. Statistical Analysis

All experiments were performed in triplicate. Data on alkaloid and terpene content, as well as extraction yield in crude extracts, were analysed using the T-student test. In contrast, data from the extract fractions were subjected to analyses of variance (ANOVA) using Statgraphics statistical software. Means were compared using Tukey´s test, with a significance level of $p \leq 0.05$ (Centurion XVI.II).

3. RESULTS and DISCUSSION

3.1. Moisture Content and Yield of Crude Extracts

The moisture content of *P. laevigata* and *V. farnesiana* leaves was determined to be 61.70 ± 1.10% and 53.12 ± 1.26% (w/w), respectively. The results showed that the moisture content in *P. laevigata* was significantly higher than that of *V. farnesiana*. In contrast, the extraction yield for both species did not exhibit a statistical difference, with values of 2.64 ± 0.42% and 2.58 ± 0.18% (w/w) for *P. laevigata* and *V. farnesiana*, respectively. The moisture content values obtained for *P. laevigata* and *V. farnesiana* leaves are comparable to those reported for other species, such as *Acacia modesta* (46.44% w/w), *Vachellia nilotica* (55.22% w/w) (Azim *et al*., 2011), and *P. juliflora* (59% w/w) (Elfadl & Luukkanen, 2003). However, the moisture content of *P. laevigata* leaves in this study was higher than the value reported by García-Azpeitia *et al*. (2022) (15.50% w/w). These discrepancies in moisture content can be attributed to differences in collection areas, seasons, and drying methodologies. In this study, freeze-drying was employed to prevent alterations in the composition of secondary metabolites, which can occur with oven-drying (ElNaker *et al*., 2021). It is essential to consider this parameter, as external factors or variables (such as light, temperature, soil water, and others) can significantly impact various processes associated with plant growth and development, including their ability to synthesize secondary metabolites. This, in turn, can lead to changes in the overall phytochemical profiles, which play a strategic role in the production of bioactive substances (Griesser *et al*., 2015).

On the other hand, the extraction process employed in this study involved biomass maceration in ethanol under constant agitation. To enhance extraction efficiency, an ultrasonic bath was utilized, followed by concentration under reduced pressure. Subsequently, a hexane extraction was performed to remove oils, waxes, and other nonpolar compounds. A second extraction with water and chloroform was then conducted to eliminate polar components, such as sugars and flavonoids. Furthermore, the chloroform extract was treated with a 1% NaCl aqueous solution to remove tannins. This extraction procedure enables the retention of most biologically active compounds from the plant sample, making it suitable for preliminary evaluation of anticancer activity, particularly for alkaloid compounds. The extraction yield values obtained in this study were comparable to those reported by Calderon-Montaño *et al*. (2021), which ranged from 1.9 to 10.7% (w/w). Their study employed a mixture of ethanol, ethyl acetate, and water as the base solvent for extraction, followed by ultrasonic extraction and evaluation of cytotoxic activity.

3.2. Alkaloid and Triterpenoid Content in Leaf Extracts

The identification of phytochemicals was performed using qualitative tests. A small amount of the dry extract was resuspended in an ethanol and chloroform mixture to observe the tests with Dragendorff's and the Liebermann-Burchard reagents for the identification of alkaloids and terpenes, respectively. Both tests were positive, indicating the presence of these phytochemicals in the extract (Fig. 1A-B). Therefore, the quantification of these compounds was carried out using spectrophotometric methods. The extract of *P. laevigata* leaves showed the highest total alkaloid content (621.64 ± 4.39 mg AE g^{-1}), whereas the *V. farnesiana* extract contained 379.84 ± 4.22 mg AE g^{-1}. In contrast, the *V. farnesiana* leaves extract exhibited the highest total triterpenoid content (364.72 ± 6.13 mg UAE g^{-1}), while the *P. laevigata* extract contained 335.60 ± 5.30 mg UAE g^{-1}.

Regarding the alkaloid content of *P. laevigata*, which was 621.64 ± 4.39 mg AE g^{-1} (equivalent to 16.53 ± 1.19 mg AE per gram of dry biomass), it was found to be higher than the value reported by Nava-Solis *et al.* (2022) for a methanolic leaf extract (11.87 mg AE per gram of dry biomass). Additionally, the alkaloid content of *P. laevigata* was also higher than that reported for *Prosopis flexuosa* (200.3 mg AE per gram of dry biomass) and *Prosopis nigra* (34.5 mg AE per gram of dry biomass) by acid/basic extraction from pulverized pods (Cholich *et al*., 2021). Similarly, the alkaloid content in *P. laevigata* was comparable to that found in *Biebersteinia multifidia* root extract (16.88 mg AE per gram of dry biomass) by Fazel *et al*.

(2010). In contrast, lower alkaloid contents were reported by Das *et al*. (2022) for *Euphorbia hirta* leaves extract (7.31 mg AE per gram of dry biomass) and by Shaik *et al*. (2011) for *Lessertia frutescens* leaves extract (4.52 mg AE per gram of dry biomass). The higher alkaloid content in the plant species studied in this work can be attributed to the use of an ultrasonic bath in the extraction process, which has been found to increase the extraction of metabolites (Rao *et al*., 2021). While the triterpenoid content in both species was similar to that reported by Rodrigues *et al*. (2021) for *Acacia dealbata* leaves (8.2 mg TT g^{-1}, total triterpenes).

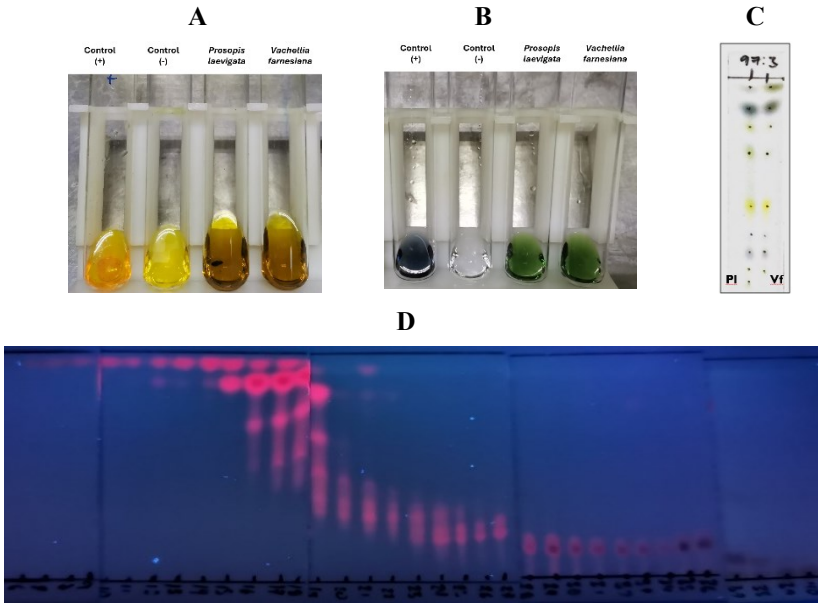

Figure 1. Qualitative analysis of plant extracts. **A)** Dragendorff´s reagent test; **B)** Liebermann-Burchard reagent test; **C**) Thin-layer chromatography (TLC) analysis of crude extracts from *Prosopis laevigata* (Pl) and *Vachellia farnesiana* (Vf), performed using aluminum plates coated with silica gel 60 F254 (Merck-Millipore) and a chloroform-methanol elution system (97:3), prior to reagent application; and D) TLC analysis of fractions obtained from the crude extract of *P. laevigata*, visualized under long-wavelength ultraviolet (UV) light (365 nm).

3.3. Fractionation of Crude Extract

The crude extracts were analyzed by thin-layer chromatography (TLC) to assess the complexity of the extract. Five solvent mixtures were tested to select the most suitable eluent for separating the bands. The 97:3 chloroform-methanol mixture was chosen due to its effectiveness in eluting the chromatoplates. The TLC plates of the leaves extract revealed nine and eight bands for *P. laevigata* and *V. farnesiana*, respectively (Fig. 1C). The bands exhibited retention factors (Rf) ranging from 0.91 to 0.06. Notably, eight bands in both species showed identical retention factors, except for one band in *P. laevigata* (Rf of 0.61). This similarity in retention factors suggests a chemical similarity between the compounds synthesized in the two species. However, identical retention factors do not necessarily indicate identical constituents. Furthermore, the intensity of the bands differed, indicating varying concentrations of these compounds in the two plant extracts. The *P. laevigata* extract showed higher intensity bands. The crude extracts were further fractionated using a chromatographic silica gel column with a mobile phase employing an elution gradient through a chloroform-methanol mixture (99:1 to 8:2 ratio). Initially, 35 fractions were obtained (Fig. 1D).

Fractions with similar chromatographic profiles were combined, resulting in eight representative fractions for each species. The fractionation results were similar to those reported by Silva *et al*. (2007) for the fractionation of the crude extract of *P. juliflora* using chromatography. Their study yielded 36 fractions, which were selected and determined using Dragendorff's reagent, and were grouped into six representative fractions based on similar

chromatographic profiles. The representative fractions from both species were analyzed separately for total alkaloid and triterpene content, as well as extraction yield. In *P. laevigata*, fraction 4 exhibited the highest total alkaloid content, with a value of 53.03 ± 0.45 mg AE g^{-1} and an extraction yield of 0.216 ± 0.030%. Notably, this fraction showed three spots on the TLC with the highest intensity. In contrast, fraction 8 displayed the highest triterpene content and extraction yield, with values of 68.93 ± 0.20 mg UAE g^{-1} and 0.705 ± 0.027%, respectively (see Table 1).

Table 1. Alkaloid and triterpenoid contents, and extraction yields of crude extract and fractions from *Prosopis laevigata* leaves.

Fraction number	Alkaloid content (mg AEg^{-1})	Triterpenoids content (mg UAEg^{-1})	Yield extraction (%, w/w)
Crude extract	621.64 ± 4.39	335.60 ± 5.30	2.640 ± 0.420
F1	3.03 ± 0.03^{f}	25.90 ± 0.11^{e}	0.113 ± 0.020^{e}
F2	16.17 ± 0.04^{c}	50.37 ± 0.39^{b}	0.165 ± 0.031^{d}
F3	9.20 ± 0.11^{e}	10.22 ± 0.18^{g}	0.060 ± 0.017^{d}
F4	53.03 ± 0.45^{a}	49.62 ± 0.23^{c}	0.216 ± 0.030^{c}
F5	10.11 ± 0.01^{d}	21.51 ± 0.64^{f}	0.147 ± 0.020^{d}
F6	0.379 ± 0.01^{g}	20.75 ± 0.32^{f}	0.141 ± 0.030^{d}
F7	23.98 ± 0.21^{b}	32.53 ± 0.26^{d}	0.303 ± 0.025^{b}
F8	10.61 ± 0.75^{d}	68.93 ± 0.20^{a}	0.705 ± 0.027^{a}

The data represent the average of three repetitions ± SD. Different letters in superscript, within each column, indicate significant differences ($p \leq 0.05$).

For *V. farnesiana*, fraction 5 showed the highest alkaloid content, with a value of 86.04 ± 1.06 mg AE g^{-1} and an extraction yield of 0.120 ± 0.023%. This fraction also exhibited the highest intensity spots on the TLC. In contrast, fraction 2 displayed the highest triterpene content, with a value of 88.37 ± 0.96 mg UAE g^{-1} and an extraction yield of 0.300 ± 0.020% (Table 2).

Table 2. Alkaloid and triterpenoid contents, and extraction yields of crude extract and fractions from *Vachellia farnesiana* leaves.

Fraction number	Alkaloid content (mg AEg^{-1})	Triterpenoids content (mg UAEg^{-1})	Yield extraction (%, w/w)
Crude extract	379.84 ± 4.22	364.72 ± 6.13	2.58 ± 0.018
F1	1.27 ± 0.06^{h}	27.28 ± 0.32^{e}	0.146 ± 0.019^{d}
F2	23.52 ± 0.81^{c}	88.37 ± 0.96^{a}	0.300 ± 0.020^{b}
F3	15.38 ± 0.81^{d}	74.80 ± 0.83^{c}	0.226 ± 0.026^{c}
F4	11.24 ± 0.10^{f}	10.42 ± 0.08^{h}	0.082 ± 0.019^{e}
F5	86.04 ± 1.06^{a}	25.85 ± 0.04^{f}	0.120 ± 0.023^{d}
F6	51.93 ± 0.11^{b}	28.91 ± 0.08^{d}	0.119 ± 0.015^{d}
F7	12.52 ± 0.18^{e}	79.07 ± 0.45^{b}	0.237 ± 0.011^{c}
F8	4.14 ± 0.05^{g}	10.66 ± 0.08^{g}	0.388 ± 0.017^{a}

The data represent the average of three repetitions ± SD. Different letters in superscript, within each column, indicate significant differences ($p \leq 0.05$).

The representative fractions showed lower complexity than the crude extract for both species. Furthermore, fractions with high alkaloid and/or triterpenes content are promising candidates for cytotoxic evaluation against cancer cell lines, given the well-documented potent biological activity and therapeutic utility of these compound groups, which have been utilized for centuries. Moreover, the rising prevalence of drug resistance in various diseases, including cancer, underscores the need for novel therapeutic agents. Meanwhile, valuable ethnobotanical knowledge of traditional medicine is being lost due to inadequate documentation (Cordell *et al*., 2001).

3.4. HPLC-Q-TOF-MS/MS Analysis of Crude Extracts

High-performance liquid chromatography (HPLC) is an ideal tool for characterizing multi-component plant extracts due to its high-resolution power, enabling rapid processing at both analytical and preparative scales. Several studies have utilized HPLC for the characterization and quantification of secondary metabolites in plant extracts, particularly phenolic compounds, terpenes, flavonoids, and alkaloids (Boligon & Athayde, 2014). Tables 3 and 4 present the tentative compounds identified in the crude extracts of *P. laevigata* and *V. farnesiana*. Notably, fatty acids such as arachidic acid (peak 7 in *P. laevigata*), octadecanoic acid (peak 6 in *V. farnesiana*), and hydroxyoctatrienoic acid (peaks 14 and 13 in *P. laevigata* and *V. farnesiana*, respectively) were detected. These compounds have been previously reported in methanol extracts of *P. juliflora* (Rajeshwaran *et al*., 2020) and *Vachellia tortilis* (Taha *et al*., 2022).

Table 3. HPLC-Q-TOF-MS of crude extracts from *Prosopis laevigata* leaves.

Peak	Time of retention (minutes)	Molecular formula	Molecular weight	$[M-H]^-$ *(m/z)*	Error (ppm)	Tentative identification	Reference
1	0.958	$C_9H_{17}NO_3$	187.1208	186.1131	0	SD	SD
2	1.175	$C_7H_6O_2$	122.0367	121.0292	-2.5	Benzoic acid	Prabha *et al*., 2018
3	1.233	$C_{21}H_{30}NO_2$	328.2276	327.2199	-0.3	SD (Flavonoid)	SD
4	1.454	$C_{24}H_{20}N_3O_4$	416.1601	415.1528	-1.2	SD	SD
5	1.516	$C_{19}H_{12}O_3$	288.0786	287.2227	-1.7	2-Hydroxy-a-naphthoflavone	Bai *et al.*, 2020
6	2.099	$C_{15}H_{10}O_5$	270.0793	269.0729	-5.2	Apigenin	Picariello *et al*., 2017
7	2.624	$C_{20}H_{40}O_2$	312.3028	311.1068	-9.3	Arachidic acid	Zhong *et al*., 2022
8	2.665	$C_{16}H_{38}N_{13}O_6$	508.3068	507.2992	-0.39	SD	SD
9	2.915	$C_{16}H_{12}O_5$	284.0684	283.0623	-6.0	SD (Flavonoid)	SD
10	3.157	$C_6H_{16}N_9O_{10}$	374.1020	373.0947	-1.3	SD	SD
11	3.331	$C_{35}H_{48}N_8O_6$	676.3696	675.3623	0.74	SD	SD
12	3.890	$C_{18}H_{16}O_7$	344.0896	343.0838	-5.8	SD (Flavonoid)	SD
13	8.212	$C_{32}H_{50}O_5$	514.3682	513.3102	0.38	3-*O*-Acetil-16-α-hydroxytrametenolic acid	Khatua *et al*., 2017
14	8.401	$C_{18}H_{30}O_3$	294.2194	293.2121	-1.7	Hydroxyoctatrienoic acid	Kiuchi *et al.,* 1997
15	27.300	$C_{19}H_{24}N_2O$	296.1888	295.1854	-1.0	Cinchonamine	Michael, 2005

Error (ppm) = (Exact mass -Calculated mass) /Exact mass x 10^6

Flavonoids, including apigenin (detected in peaks 6 and 7 of *P. laevigata* and *V. farnesiana*, respectively), were identified, along with proposed molecular formulas corresponding to flavonoid family isomers (peaks 3, 5, 9, and 11 in *P. laevigata*; peaks 3 and 5 in *V. farnesiana*). Notably, apigenin has been increasingly recognized as a cancer chemopreventive agent and exerts anti-tumor effects primarily through apoptosis and cell-cycle arrest induction (Singh *et al*., 2022). Furthermore, these compounds have been previously reported in *P. laevigata* (García-Andrade *et al*., 2013) and identified in *P. juliflora* (Sathiya & Muthuchelian, 2011).

A triterpenoid derived from tramethenolic acid, specifically 3-O-acetyl-16-α-hydroxytrametenolic acid, was identified in peaks 13 and 12 of P. *laevigata* and *V. farnesiana,* respectively. This compound is classified as a lupeol derivative, as previously reported by Liu *et al*. (2021). Tramethenolic acid has been demonstrated to exhibit potent cytotoxic activity against breast and gastric cancer cells, primarily through inhibition of the P-glycoprotein transporter and suppression of H+/K+-ATPase activity. Moreover, tramethenolic acid has been

found to display synergism with other anticancer agents, such as Taxol (Dilyana *et al*., 2021). In addition to tramethenolic acid, other terpenes have been detected in *Prosopis* species. For example, Khalid et al. (2024) identified squalene and (3α)-(lupeol acetate) in ethanol extracts of *P. juliflora*, which exhibited cytotoxic activity against MCF-7, A2780, and HT-29 cell lines. Similarly, Elshikh *et al.* (2025) reported the presence of a diterpenoid, (1S,3E,4S,5R,7E,11E)-cembra-2,7,11-trien-4,5-diol, in ethanol extracts of *Vachellia flava*.

Table 4. HPLC-Q-TOF-MS of crude extract from *Vachellia farnesiana* leaves

Peak	Time of retention (minutes)	Molecular formula	Molecular weight	$[M-H]^-$ *(m/z)*	Error (ppm)	Tentative identification	Reference
1	0.943	$C_9H_{17}NO_3$	187.1208	186.1129	-0.5	SD	SD
2	1.197	$C_7H_5O_3$	137.0239	136.0171	7.4	Salicylic acid	Manríquez-Torres *et al*., 2020
3	1.480	$C_{18}H_{10}NO_2$	272.0711	271.0633	0.0	SD (Quinoline)	SD
4	1.496	$C_{15}H_{10}O_5$	270.0528	269.0458	3.0	SD (Flavonoid)	SD
5	1.509	$C_{21}H_{30}NO_2$	328.2276	327.2181	-5.2	SD (Flavonoid)	SD
6	1.825	$C_{18}H_{30}O_4$	310.2144	309.2072	1.9	Acid octadecenoic	Ali *et al.*, 2021
7	1.996	$C_{15}H_{10}O_5$	270.0793	269.0729	5.2	Apigenina	Picariello *et al*., 2017
8	2.125	$C_{19}H_{24}N_4$	308.2000	307.1920	-0.7	SD (Quinoline)	SD
9	2.975	$C_{19}H_{22}N_4$	306.1844	305.1761	-1.6	SD (Quinoline)	SD
10	5.556	$C_{19}H_{28}N_4$	312.2313	311.2235	0	SD (Quinoline)	SD
11	6.510	$C_{18}H_{28}O_3$	292.2038	291.1978	6.2	SD	SD
12	8.315	$C_{32}H_{50}O_5$	514.3682	513.3102	9.1	3-*O*-Acetil-16-α-hydroxytrameten olic acid	Khatua, *et al*., 2017
13	8.371	$C_{18}H_{30}O_3$	294.2194	293.2130	4.8	Hydroxyoctatrien oic acid	Kiuchi *et al.,* 1997
14	27.331	$C_{19}H_{24}N_2O$	294.1732	293.1627	-9.2	Cinchonine	Arumugam *et al.,* 2024

Error (ppm) = (Exact mass -Calculated mass) /Exact mass x 10^6

Alkaloids derived from quinoline (detected in peaks 3, 8, 9, and 10 in *V. farnesiana*), cinchonine (peak 14 in *V. farnesiana*), and cinchonamine (peak 15 in *P. laevigata*) were identified. Quinoline serves as a promising scaffold for anticancer drug development, as its derivatives have demonstrated potent antitumor effects through various mechanisms, including apoptosis, disruption of cell migration, inhibition of angiogenesis, modulation of nuclear receptor responsiveness, and cell cycle arrest. The potential of quinoline derivatives has been validated in several cancer cell lines, including breast, colon, lung, colorectal, and renal cancer (Ilakiyalakshmi & Arumugan, 2022). Moreover, cinchonine has been demonstrated to significantly inhibit cancer cell growth, migration, and invasion *in vitro*, as well as tumor growth and metastasis *in vivo*, without apparent toxic effects (Wang *et al*., 2023). Additionally, studies by Kacprzak *et al.* (2018) and Qi *et al.* (2017) determined the cytotoxic effects of cinchonine-family alkaloids, which exhibited cytotoxic effects against HeLa and A549 cells among other cancer cell lines. Notably, the crude extracts' peak profiles exhibited similar retention times, confirming the presence of alkaloid and triterpene groups. Several of these compounds and their derivatives have been linked to inhibiting cancer cell proliferation in previous reports.

Previous studies reported the presence of cinchona family alkaloids in *Prosopis* genus extracts (Michael, 2005) and *V. farnesiana* root extracts (Haque *et al*., 2012). The presence of benzoic acid (peak 2 in *P. laevigata*) was also detected, which has been previously reported in this plant

species, specifically in mesquite gum (López-Franco *et al*., 2021). Additionally, salicylic acid (peak 1 in *V. farnesiana*) was identified, consistent with previous findings in the leaves of this species (Manríquez-Torres *et al*., 2020). Molecular formulas and weights were proposed for the detected compounds based on exact molecular weights obtained through HPLC. However, further analysis is required for certain compounds, including peaks 1, 4, 8, 10, and 11 in *P. laevigata* and peaks 1 and 11 in *V. farnesiana*, due to insufficient spectral data matching previously reported spectra.

4. CONCLUSION

The extraction methodology employed in this study for *Prosopis laevigata* and *Vachellia farnesiana* successfully yielded extracts rich in phytochemicals with similar polarity to chloroform, predominantly alkaloids, at high concentrations. Triterpenes were also detected in the extract, albeit at lower concentrations. Notably, the crude extract contained alkaloids from the cinchonine family, as well as 3-*O*-acetyl-16-α-hydroxytrametenolic acid, a cytotoxic triterpenoid. Both plant species yielded fractions with promising potential for the development of novel anticancer therapeutics, characterized by significant alkaloid and triterpene content. These findings warrant further investigation through cytotoxicity assays in cancer cell lines to explore their therapeutic potential.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

E. H-M.: Investigation, formal analysis, and writing-original draft. **C. G-M.**: Methodology, review, and editing. **J. O-V.**: Methodology, resources, validation, review, and editing. **L. B-G.**: Conceptualization, investigation, methodology, resources, formal analysis, writing-original draft, review, and editing

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