Natural Resources for Human Health

Antibacterial, phytochemical and GC-MS analysis of *Thevetia peruviana* extracts: An approach in drug formulation

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ABSTRACT: *Thevetia peruviana* is a large flowering shrub in the Apocynaceae family used medicinally. The current study aims to determine the phytochemicals and antimicrobial potential of *T. peruviana*. GC-MS was used to screen the ethanol and n-hexane extracts. Leaf extracts (ethanol and hexane) revealed alkaloids, phenols, anthraquinones, cardiac glycosides, flavonoids, tannins, steroid glycosides, carbohydrates, proteins, terpenoids, terpenoids and fixed oils and fats. GC-MS analysis of the ethanol and hexane extract revealed 24 bioactive metabolites. *T. peruviana* leaf extracts inhibited tested pathogens at 50, 100, and 200 mg/ml concentrations. A plethora of secondary metabolites demonstrated promising pharmacological benefits. The bioactive chemicals are utilised to treat bacterial infections, cancer, diabetes, and inflammation. This study demonstrated the antibacterial activity of several plants used in traditional medicine.

1. INTRODUCTION

Plants can generate a tremendous amount of numerous bioactive compounds like alkaloids, essential oils, terpenoids, gums, resins, flavonoids, etc., that have already been discovered to acquire biological activities against pathogenic microbial organisms (Mansoori et al., 2020). Vegetable products have been a part of herbal medicines that can be obtained from every part of the plant, such as its seeds, flowers, bark, leaves, and roots. Vegetable products offer vital chemical variety to modern-day medicine exploration programs. Playing the part of conventional medicine in solving health issues is priceless at the global level.

Plant-based medicines have become popular because green medicines are secure and readily accessible with fewer or no adverse effects. Undeniably, the marketplace and demand in the community have proved to be so good that there is a high risk that many medicinal plants at present face either extinction or loss of genetic variation. Understanding the chemical components of plants remains suitable because this information will be beneficial to producing complex biochemical substances and helping the scientific foundation of the therapeutic effect of these green medications.

Thevetia peruviana, an active member of the Apocynaceae family, popularly known as Yellow oleander, remains an ornamental plant growing all around Africa, Australia, and Asia. An oil-based dye that contains the species has been reported to safeguard timber since it displays antibacterial, anti-inflammatory, and anti-termite characteristics (Echeverri et al., 2019) (Bado et al., 2015). The colloidal impact of its fluid, stem bark and leaf has also been reported (Hassan et al., 2011). Its seed and oil have been reported in connection with their possibilities for agriculture and industrial use (Drzewoski & Hanefeld, 2021). These plants developed biologically active compounds manufactured at the same time as secondary metabolites and continue to be stored in different plant parts. The existence of such phytochemicals is a sign that the plant may also be a potential store of the following foundations for the development of herbal preparation (Echeverri et al., 2019). Previously, the occurrence of essential phytochemicals such as alkaloids, terpenoids, anthraquinones, flavonoids, cardiac glycosides and tannins have been reported (Kadiri & Olawoye, 2016). Likewise, a high level of protein and vital micronutrients have been discovered in this species and have been discovered to be anti-sickling in the action (Jing et al., 2016).

It was observed that the plant is being used in the treatment of several diseases such as gonorrhoea, skin diseases and diarrhoea (Daswani et al., 2017). As a supplement, its antibacterial and gastrointestinal protective characteristics, along with antioxidant antidiabetic and hematinic characteristics, have already been reported (Akhbari et al., 2019).

Microbial infectious diseases continue to remain the foundation for substantial morbidity and mortality worldwide, frequently caused by medication failure or treatment option limitations on the occurrence of resistant isolates of antimicrobial therapy (Jing et al., 2016). Plant-based antimicrobial products correspond to an immense available resource for medicinal products. Several biological activities of *Thevetia peruviana* leaves such as anti-inflammatory (Rai et al., 2019), antidiarrhoeal, anti-microbial and cytotoxic (Petronzi et al., 2013), anti-spermatogenic (Ahsan et al., 2017), piscicidal (Özçelik et al., 2005) and anti-termite (Sham et al., 2013) have been reported. The current work aimed to identify the phytochemicals and microbial evaluation of the leaf extracts of *Thevetia peruviana*. Gas chromatography-mass spectroscopy was used to identify the extracts' bioactive compounds.

2. MATERIALS AND METHODS

2.1. Crude extract preparation

Thevetia peruviana Leaves (Figure 1) were collected in its natural growing environment in Lefkosia, Cyprus. The plant was presented for recognition at the Department of Agriculture, Cyprus International University. The plant was dried at ambient temperature and grounded in an electrical mill. The grounded raw material was passed via a weave filter size of 60 mm to obtain a fine powder. It was subsequently used to prepare the extracts (n-hexane and ethanol). The Crude extracts were prepared in compliance with the cold extraction method. Furthermore, 30 grams of dried powder was soaked in 300 ml of ethanol and hexane respectively and kept for 48 hours. After 48 hours, the mixtures were filtered utilizing the Whatman filter paper and dried in the vacuum using the rotary vacuum evaporator (Barupal et al., 2019).

Figure 1. *Thevetia peruviana* leaves

2.2. Chemicals and materials

The chemicals used were all analytical grade reagents. The chemicals used were purchased from the Sigma-Aldrich chemical company (St. Louis, MO, USA). High-purity culture media were delivered by Merck (India). The Milli-Q purification system (Millipore, Bedford, MA, USA) was used to refine the water used in the research analysis.

2.3. Phytochemical analysis

Qualitative analysis has been designed to detect various secondary metabolites or phytochemicals present in extracts. The extracts were subjected to a qualitative test proposed by (Rai et al., 2019) (Altemimi et al., 2017).

2.4. Collection of Clinical pathogens

The following clinical pathogens, including *Escherichia coli*, *Salmonella* species, *Shigella* species and *Staphylococcus aureus*, were obtained from the Department of Biochemistry for this study.

2.5. Culture media preparation and inoculums standardization

The McFarland nephelometer technique was used to standardise the test organisms (Zapata & Ramirez-Arcos, 2015). The test microorganisms were transferred to sterile Nutrient broth (10 ml) and cultured at 37 °C for 18 h. The turbidity produced after 18 was simultaneously adjusted to fit McFarland standards (0.5 %) by adding sterile nutrient broth to cultured samples and comparing the results using a nephelometer to McFarland standards (0.5 %).

2.6. Antibacterial Assay

The antibacterial activity of the extracts was determined using the agar well diffusion technique (Ibrahim et al., 2018). Each well (100 l) includes 50 mg/ml, 100 mg/ml, and 200 mg/ml of ethanol and n-hexane leaf extracts of *T. peruviana* that have been dissolved in DMSO (dimethyl sulfoxide) (5%) and distributed uniformly in four wells. Three duplicates of each test were conducted.

2.7. Susceptibility of an isolated organism with antibiotics

According to the manufacturer's instructions, Mueller-Hinton agar was prepared and allowed to cool. The media was dispensed aseptically and allowed to solidify on sterile Petri dishes. Additionally, using sterile swab sticks, 0.1 ml of the standardised inoculum was dispersed equally across the surface of the prepared solid medium. Antibiotics (Augmentin$_{(ab)}$ = 30μg, Ofloxacin$_{(ab)}$ = 5μg, Ciprofloxacin$_{(ab)}$ = 5μg, Nitrofurantoin 300μg, CXM$_{(ab)}$ = Cefixime 5μg, Gen = Gentamycin$_{(ab)}$ = 10μg, Cefuroxime$_{(ab)}$ = 30μg, Ceftazidime$_{(ab)}$ = 30μg) was applied to the surface of seeded organisms in petri dishes and incubated at 37 °C for 24 h. Antibiotics were quantified in μg. The maximal diameter of inhibitory zones across each disc was measured millimetres following incubation.

2.8. GC-MS analysis

The active metabolites in ethanol and n-hexane extracts was determined by GC–MS (SHIMADZU QP2010). Hexane extract (1 μl) was added to the GC. The capillary column

(30 m x 0.25 m x 0.25 m) was used in: Injector temperature (250 °C), carrier gas (helium), flow rate 1 ml/min; injection sample volume 1 μl; split ratio 1:0; ionisation energy 70 eV: Run duration 28 min. Each metabolites' relative amount was calculated by comparing its average peak area to the overall area. Identifying the isolated volatile metabolites was done using retention indices and mass spectrometry with the NIST library 2008 database.

2.9. Statistical analysis

All the analysis was done in triplicate (n = 3) as well as all results were expressed as mean ± standard.

Statistical analysis of collected data (ethanol and hexane extract) was performed using IBM SPSS Statistics 23 software. Using Duncan's multiple comparison tests, the significance threshold was chosen at P<0.05.

3. RESULTS AND DISCUSSION

The usage of medicinal plant extracts for several illnesses, such as cancer therapy, is fast developing since they are affordable along with limited or any side effects. The active substances in the extracts have effectively prevented diseases (Rai et al., 2019). The antibacterial activity demonstrated by the examined ethnobotanicals could be attributed to the presence of a variety of phytochemicals such as terpenoids and alkaloids (Mujeeb et al., 2014). The existence of flavonoids in the leaves of *Thevetia peruviana* might be responsible for its antimicrobial activity due to their ability to intricate with the cell walls of bacterial (Tsuchiya et al., 1996). Ethanol and n-hexane extracts demonstrated the most appropriate antibacterial activity (Tables 1 and 2). n-Hexane extracts at marked concentration (200 mg/ml) demonstrated activity against *Shigella* species (20.01 ± 2.00), *Salmonella* species (19.09 ± 0.50), *Staphylococcus* aureus (19.50 ± 0.25) and *Escherichia* coli (20.01 ± 0.50) (Table 2). The highest zone of inhibition (200 mg/ml) was significantly higher in some test organisms in comparison to the zone of inhibitions shown by the standard antibiotics drugs, which are Ceftazidime, Cefuroxime, Gentamycin (i.e., *Staphylococcus aureus*), Nitrofurantoin (except *Escherichia coli*), Cefixime and Augmentin (Table 4). n-Hexane extract revealed a resistance diameter of 19.3 mm for *Staphylococcus aureus* and inhibition diameters of *Salmonella* species (23 mm), respectively (Echeverri et al., 2019). Ethanol extract has demonstrated the greatest inhibition against *Shigella* species (22.51 ± 0.49), *Salmonella* species (20.10 ± 0.39), *Staphylococcus aureus* (24.96 ± 0.50) and *Escherichia coli* (24.57 ± 2.01) (Table 1). The ethanol extract of *Thevetia peruviana* leaves indicates that the concentration depends on the antibacterial activity of *Staphylococcus aureus* and *Escherichia coli* (Kareru et al., 2010). The maximum inhibition diameters of *Escherichia coli* (10.33 and 9.33 mm) reported by (Echeverri et al., 2019) were lesser than the present results. The antibacterial activity of *Staphylococcus aureus* on ethanol leaves extract has an inhibition diameter of 15 mm reported by (Echeverri et al., 2019) were

lesser than the present results. Both extracts revealed resistance against *E. coli*. Likewise, (Mujeeb et al., 2014) stated meagre inhibition rates for ethanol extracts of leaves at 100 mg/ml concentrations on bacteria, particularly *Staphylococcus aureus* (2.7 mm). However, despite the higher zones of inhibition demonstrated in ethanol extracts of *Thevetia peruviana* leaves, the highest zone of inhibition (200 mg/ml) were significantly higher in some test organism in the comparison to zone of inhibitions shown by the standard antibiotics drugs which are Ceftazidime, Cefuroxime, Gentamycin (i.e, *Staphyllocuccus aureus*), Nitrofurantoin, Cefixime and Augmentin (Table 4). Additional authors have stated the antibacterial activity of extracts derived from the leaves of *Thevetia peruviana*, achieving extremely good results against bacteria and fungi for ethanol and hexane extracts (Deshmukh et al., 2019) (Evbuomwan et al., 2018). Ethanol and hexane extracts revealed a more inhibitory impact on the inhibition growth of bacteria, which is consistent with reports that have been described above. Results that have been achieved in this work for ethanol and hexane extracts are quite similar to those described by some of the already mentioned authors.

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Table 1

Zones of inhibition of ethanol extract of *Thevetia peruviana* leaves Crude extract against some bacteria

Concen. (mg/mL)	*Shigella* species	*Salmonella* species	*Staphyllocuccus aureus*	*Escherichia coli*
Zones of inhibition (mm)				
50	10.00 ± 0.57^a	14.10 ± 0.25^a	14.09 ± 1.25^{ab}	16.01 ± 2.50^{ba}
100	16.50 ± 0.51^b	14.20 ± 0.31^a	19.75 ± 0.26^{ab}	19.67 ± 2.69^{ba}
200	22.51 ± 0.49^c	20.10 ± 0.39^b	24.96 ± 0.50^b	24.57 ± 2.01^b

Values in each column are presented as mean ± SD (i.e. n = 3). Using One-way ANOVA
Means in the same column with a different superscript are significantly different (p < 0.05).

Table 2

Zones of inhibition of n-hexane extract of *Thevetia peruviana* leaves Crude extract against some bacteria.

Concen. (mg/mL)	*Shigella* species	*Salmonella* species	*Staphyllocuccus aureus*	*Escherichia coli*
Zones of inhibition (mm)				
50	13.01 ± 1.06^a	14.05 ± 0.50^a	14.03 ± 1.25^{ab}	14.01 ± 1.06^a
100	17.11 ± 0.25^b	15.01 ± 0.75^a	16.04 ± 1.00^{ab}	18.55 ± 2.75^{bb}
200	20.01 ± 2.00^b	19.09 ± 0.50^b	19.50 ± 0.25^b	20.01 ± 0.50^{bb}

Values in each column are presented as mean ± SD (i.e. n = 3). Using One-way ANOVA
Means in the same column with a different superscript are significantly different (p < 0.05).

Qualitative analysis of several secondary metabolites was examined in ethanol and hexane extracts. Usually, the therapeutic properties of medicinal plants could be attributed

Table 3

Minimal inhibitory concentrations (MIC) of ethanol and n-hexane extract of *Thevetia peruviana* leaves against some bacteria.

Test organisms	MIC (mg/mL)	
	Ethanol	*n*-hexane
Shigella species	50	50
Salmonella species	50	50
Staphylococcus aureus	50	50
Escherichia coli	50	50

Table 4

Resistance of bacteria with various antibiotics.

Pathogens	Cef-tazidime	Cefuroxime	Gen-tamycin	Ciprofloxacin	Nitro-fu-ran-toin	Cefixime	Ofloxacin	Aug-mentin
Zones of inhibition (mm)								
Shigella species	15	15	24	25	15	5	26	13
Salmonella species	14	16	26	26	15	6	25	1\4
Staphy-lococcus aureus	15	15	14	25	16	5	24	13
Escherichia coli	14	16	26	26	20	6	28	14

Table 5

Qualitative analysis of the extract of *Thevetia peruviana*

| Phytochemicals	Name of test	*Thevetia peruviana*	
		Ethanol	n-hexane
Alkaloids	Mayer's test	+	+
	Dragendroff's test	+	+
Saponins	Frothing test	-	-
Tannins	Lead acetate	+	+
	Shinoda test	+	+
Flavonoids	Alkaline reagent test	+	+
	Sodium hydroxide test	+	+
Phenols	Phenol reagent	+	+
Anthraquinones	Modified Bontrager's test	+	+
Cardiac glycosides	Liebermann's test	+	+
	Keller-kilani test	+	+
Steroids	Steroids	+	+
Carbohydrates	Fehling's test	+	+
	Iodine Test	+	+
Proteins	Million's test	+	+
Terpenoids		+	+
Fixed oils & fats	Stain test	+	+
Resins	Acetone-water test	-	-

Keys: + (present), - (absent)

to the existence of various secondary metabolites such as glycosides, flavonoids, alkaloids, sterols etc. Hence, the preliminary screening is vital for discovering chemical groups that could further lead to compounds' characterisation. All these identified phytochemicals have various biological activities, including antiviral, antioxidant, cytotoxic, antifungal, and antibacterial activities (Larayetan et al., 2019). The findings revealed the presence of alkaloids, flavonoids, tannins, phenols, anthraquinones, cardiac glycosides, steroids, carbohydrates, proteins, terpenoids, fixed oils & fats in ethanol and hexane extracts (Table 5).

Similarly, saponins and resins were not present in both sample extracts. These phytochemicals provide great pharmacological activities in traditional medicine, which might be responsible for the improved activity of the extract, as shown in Table 5 . For n-hexane extract, the MICs of the extract were 50 mg/mL against *Shigella* species, *Salmonella* species, *Staphylococcus aureus* and *Escherichia coli*. The ethanol extract had MICs of 50 mg/mL against *Shigella* species, *Salmonella* species, *Staphylococcus aureus* and *Escherichia coli*. (Table 3). The therapeutic and pharmacological effects of the plant are often dependent on the existence of its secondary metabolites (Gorlenko et al., 2020). Following the preliminary phytochemical analysis, ethanol and hexane extracts contain secondary metabolites such as glycosides, flavonoids, alkaloids, sterols and terpenoids (Onyibe et al., 2021). Alkaloids constitute naturally occurring nitrogen-containing organic compounds with antibacterial characteristics (Dey et al., 2020). Flavonoids and tannins are the leading phenolic compounds that act as anticarcinogenic, antimutagenic activity, antioxidants, and anti-inflammatory properties (Dey et al., 2020). Terpenoids are being used for hepatoprotective, antipyresis, antidiabetic, pain-relieving and soothing (Özçelik et al., 2005). The plant *Thevetia peruviana* (S) has traditionally been utilised for fever medication as a nauseant, laxative, and antitumor treatment. Phytochemistry study of *Thevetia peruviana* leaves has been revealed for its richness in bioactive compounds (El-Sawi et al., 2020). The leaves of this plant have also been reported to possess great therapeutic value, as well as in phytochemical investigation revealed the presence of glycosides, alkaloids, tannins, carbohydrates, fixed oils, proteins and sterols (Mujeeb et al., 2014). Although the leaves and stems are currently being used for a small number of topical applications within traditional medicine, the lack of scientific reports about the leaf urged us the opportunity to carry out a methodical phytochemistry analysis of the plant (Nwosu et al., 2022). Mainly, organoleptic research of plant raw material has assisted in the confirmation of certain organic compounds owing to the aromatic scent of extracts from the sweet flavor showing the presence of sugar in the plant (Edo, 2022). Future investigations will make it possible to throw much light on the favourable properties, which might open up new avenues to efficiently utilize the plant as a rich source of bioactive compounds in the pharmaceutical industry (Hassan et al., 2021).

The ethanol and hexane extracts were subjected to GC-MS to identify the phytochemical constituents. Figure 2 and 3 were the chromatogram of ethanol and hexane extracts. In each extract, the phytocompounds are said to have been present between retention times 3.508 to 26.500 and 3.842 to 27.042, respectively. In hexane extract, ten biologically active compounds were identified, and these biologically active compounds names, retention time, area, mole formula, mole weight, PubChem CID and their bio-active uses were tabulated in Table 6. The biologically active compounds are Hexadecanoic acid, 9,12,15-Octadecadienoic acid, Ethyl tridecanoate, Dodecanoic acid, Tetradecanoic acid, Eicosane, Undecanoic acid, Farnesol, Squalene and 2,5-Cyclohexadiene-1,4-dione have been reported as hypocholesterolemic, antimicrobial, antitumor, immunosuppressant, antioxidant, nematicide and antiproliferative agent.
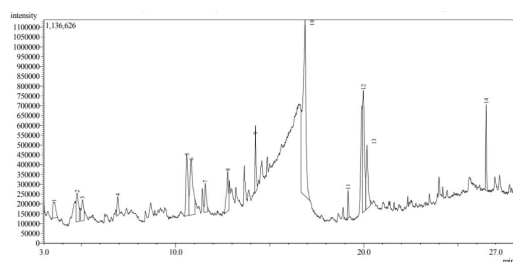
Figure 2. GC-MS chromatogram of ethanol extract of *Thevetia peruviana* Leaves.
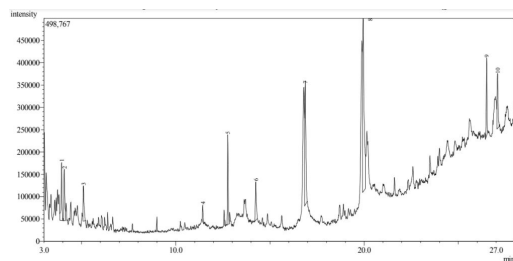
Figure 3. GC-MS chromatogram of n-hexane extract of *Thevetia peruviana* Leaves.

In ethanol extract, fourteen biologically active compounds have been identified, and these biologically active compounds names, retention time, area, mole formula, mole weight, PubChem CID and their bio-active uses were tabulated in Table 7. The biologically active compounds are 2H-Pyran-2-one, 2(3H)-Furanone, Nonadecanoic acid, 9,12-Octadecadienoic acid, Nonadecanoic acid, 2,5-Cyclohexadiene-1,4-dione, 3-O-Benzyl-d-glucose, Docosanoic acid, (Z)-Octadec-9-enoic acid, 13-Docosenoic acid, Hexadecanoic acid, Farnesol, Squalene, Pentadecanoic acid has been reported as hypocholesterolemic, antimicrobial,

antitumor, immunosuppressant, antioxidant, nematicide, antiproliferative, anticholinesterase, radioprotective, antifertility, antihyperlipidaemic, repellents, pharmaceutical agent, emollient, 5-Alpha reductase inhibitor and antidiabetic property.

Under the current research, compounds of ethanol and hexane crude extracts were identified with compound name, molecular weight, and molecular formula through GC-MS evaluation. GC-MS identified several phytocompounds from ethanol and hexane extracts in the present study. Ten bioactive compounds were identified in hexane extract. The bioactive compounds are Hexadecanoic acid with a retention time of 3.938 and peak area of 7.24, has antimicrobial, antioxidant, antifungal, solvent, hypocholesterolemic, emollient, anti-inflammatory, pharmaceutical agent and 5-Alpha reductase inhibitor (Nalawade et al., 2015). 9,12,15-Octadecadienoic acid has a retention time of 4.08, and a peak area of 6.78 is used as an Anti-inflammatory, anti-asthma, antimicrobial, antioxidant, anticancer, anti-arthritic, Hepatoprotective and diuretic (Salah et al., 2015). Ethyl tridecanoate, with a retention time of 5.096 and a peak area of 4.3, is used as antioxidant, antimicrobial and anticancer activities (Usman et al., 2018). Dodecanoic acid, with a retention time of 11.432 and a peak area of 1.37, is used as an antibacterial and antifungal agent (Seidel & Taylor, 2004). Tetradecanoic acid has a retention time of 12.76, and a peak area of 8.57 is used as antibacterial, lubricant and nematicide (Salehi et al., 2019). Eicosane, with a retention time of 14.247 and a peak area of 4.96, has Antipyretic, antioxidant, anti-inflammatory and antifungal (Ahsan et al., 2017). Undecanoic acid has a retention time of 16.876, and a peak area of 17.93 is used as antioxidant, antifungal and anticancer activity (Rai et al., 2019). With a retention time of 19.945 and a peak area of 33.88, Farnesol is used for anticancer, anti-inflammatory and immunosuppressant activity (Jung et al., 2018). Squalene with a retention time of 26.502 and peak area of 7.69 is used as Antimicrobials, antitumor agents, supplements, antioxidants, anti-cancer, repellents and hypocholesterolemic (Gnes, 2013) (Lozano-Grande et al., 2018). 2,5-Cyclohexadiene-1,4-dione has a retention time of 27.079, and a peak area of 7.28 is used as Antitumor activity and antiproliferative agent (Petronzi et al., 2013). Likewise, in ethanol extract, fourteen known bioactive compounds have been identified, and they are 2H-Pyran-2-one with a retention time of 3.508 and peak area of 2.66, has Antibacterial, anticancer agent, antifungal activity (Raynor et al., 2004). 2(3H)-Furanone has a retention time of 4.764, and a peak area of 3.76 is used as Antibacterial, hematopoietic, hepato-irritant, antioxidant and hyperthermic (Oni et al., 2020). Nonadecanoic acid with the retention time at 5.051 and peak area of 2.84 is used as Anticancer, anti-obesity, antioxidant and antitumor (Gao et al., 2012). 9,12-Octadecadienoic acid with a retention time of 6.922 and peak area of 1.70 is used for Hepatoprotective, anti-inflammatory, antihistaminic, hypocholesterolemic, anti-eczemic and anti-arthritic activities (Sofowora et al., 2013). Decanoic acid has a retention time of 10.597, and a

Table 6
Biological activity of n-hexane extract-identified metabolites.

Peak	Compounds	R.Time	Area %	Mole Formula	Mole Weight (g/mol)	PubChem CID	Bioactive uses
1	Hexadecanoic acid	3.938	7.24	C16H32O2	256.42	985	Antimicrobial, antioxidant, antifungal, solvent, hypocholesterolemic, emollient, anti-inflammatory, pharmaceutical agent and 5-Alpha reductase inhibitor (Nalawade et al., 2015).
2	9,12,15-Octadecadienoic acid	4.08	6.78	C18H32O2	280.4	3931	Anti-inflammatory, anti-asthma, antimicrobial, antioxidant, anticancer, anti-arthritic, Hepatoprotective and diuretic (Salah et al., 2015).
3	Ethyl tridecanoate	5.096	4.3	C15H30O2	242.4	119908	Antioxidant, antimicrobial and anticancer activities (Usman et al., 2018).
4	Dodecanoic acid	11.432	1.37	C12H24O2	200.32	3893	Antibacterial and antifungal agents (Seidel & Taylor, 2004).
5	Tetradecanoic acid	12.76	8.57	C14H28O2	228.37	11005	Antibacterial, lubricant and nematicide (Salehi et al., 2019).
6	Eicosane	14.247	4.96	C20H42	282.5	8222	Antipyretic, antioxidant, anti-inflammatory and antifungal (Ahsan et al., 2017).
7	Undecanoic acid	16.876	17.93	C11H22O2	186.29	8180	Antioxidant, antifungal and anticancer activities (Rai et al., 2019).
8	Farnesol	19.945	33.88	C15H26O	222.37	445070	Anticancer, anti-inflammatory and immunosuppressant activities.
9	Squalene	26.502	7.69	C30H50	410.7	638072	Antimicrobials, antitumor agent, supplements, antioxidant, anti-cancer, repellents and hypocholesterolemic (Lozano-Grande et al., 2018).
10	2,5-Cyclohexadiene-1,4-dione	27.079	7.28	C9H10O2	150.17	70291	Antitumor activity and antiproliferative agent (Petronzi et al., 2013).

peak area of 7.52 is used as anticancer, antibacterial, anti-inflammatory and hypocholesterolemic (Lee et al., 2021). 2,5-Cyclohexadiene-1,4-dione with a retention time of 10.819 and peak area of 10.37 has Antitumor activity and antiproliferative agent (Petronzi et al., 2013). 3-O-Benzyl-d-glucose has a retention time of 11.578 and a peak area of 2.94 is used as an antioxidant, anticholinesterase, radioprotective, antimicrobial, antifertility, antihyperlipidaemic and antidiabetic property. Docosanoic acid with the retention time at 12.764 and peak area of 4.64 is used as Anthelminthic, hypoglycemic and antihepatotoxic (Petronzi et al., 2013). (Z)-Octadec-9-enoic acid with the retention time at 14.244 and peak area of 3.72 is used as Anti-inflammatory, antimicrobial and antioxidant properties (Lin et al., 2017). 13-Docosenoic acid has retention time at 16.877 and peak area of 33.56 is used as Antimicrobial, antioxidant and hypocholesterolemic (Sham et al., 2013), Hexadecanoic acid with the retention time at 19.162 and peak area of 1.58 has Antimicrobial, antioxidant, antifungal, solvent, hypocholesterolemic, emollient, anti-inflammatory, pharmaceutical agent and 5-Alpha reductase inhibitor (Nalawade et al., 2015). Farnesol has the retention time at 19.971 and peak area of 13.48 is used as Anticancer, anti-inflammatory and immunosuppressant activities (Jung et

al., 2018). Squalene with the retention time at 26.502 and peak area of 7.69 is used as Antimicrobials, antitumor agent, supplements, antioxidant, anti-cancer, repellents and hypocholesterolemic (Gnes, 2013) (Lozano-Grande et al., 2018). Pentadecanoic acid with the retention time at 26.5 and peak area of 4.09 is used as Antioxidant, antifungal and antimicrobial activities (Oni et al., 2020). Thus, the current analysis is believed to aid in identifying antioxidant and cytotoxicity activities of ethanol and hexane extracts.

4. CONCLUSION

Thevetia peruviana leaves an abundant source of phytochemicals such as cardiac glycosides, anthraquinone, alkaloids, flavonoids, tannins, and phenols. The existence of the constituents mentioned above all together could be attributed to pharmacological characteristics. An abundance of secondary metabolites could undoubtedly encourage results in further pharmacological activities. The bioactive compounds are used for different diseases such as bacterial infection, cancer, diabetes and inflammation. Therefore, the bioactive compounds will be generated in substantial quantities via micropropagation.

Additionally, substantial antioxidant and cytotoxicity activities have been reported in this study, and the findings

Table 7
Biological activity of ethanol extract-identified metabolites.

Peak	Compounds	R.Time	Area%	Mole Formula	Mole Weight (g/mol)	Pub chem CID	Bioactive uses
1	2H-Pyran-2-one	3.508	2.66	C5H4O2	96.08	68154	Antibacterial, anticancer agent and antifungal activity (Raynor et al., 2004).
2	2(3H)-Furanone	4.764	3.76	C4H4O2	84.07	140765	Antibacterial, hematopoietic, hepato-irritant, antioxidant and hyperthermic (Oni et al., 2020).
3	Nonadecanoic acid	5.051	2.84	C19H38O2	298.5	12591	Anticancer, anti-obesity, antioxidant and antitumor (Gao et al., 2012).
4	9,12-Octadecadienoic acid	6.922	1.70	C18H32O2	280.4	3931	Hepatoprotective, anti-inflammatory, antihistaminic, hypocholesterolemic, anti-eczemic and anti-arthritic activities (Sofowora et al., 2013).
5	Decanoic acid	10.597	7.52	C10H20O2	172.26	2969	Anticancer, antibacterial, anti-inflammatory and hypocholesterolemic (Lee et al., 2021).
6	2,5-Cyclohexadiene-1,4-dione	10.819	10.37	C9H10O2	150.17	70291	Antitumor activity and antiproliferative agent (Petronzi et al., 2013).
7	3-O-Benzyl-d-glucose	11.578	2.94	$C_{13}H_{18}O_6$	270.28	10978515	Antioxidant, anticholinesterase, radioprotective, antimicrobial, antifertility, antihyperlipidaemic and antidiabetic property (Gnes, 2013).
8	Docosanoic acid	12.764	4.64	C22H44O2	340.6	8215	Anthelminthic, hypoglycemic and antihepatotoxic (Sham et al., 2013),
9	(Z)-Octadec-9-enoic acid	14.244	3.72	C18H34O2	282.5	445639	Anti-inflammatory, antimicrobial and antioxidant properties (Lin et al., 2017).
10	13-Docosenoic acid	16.877	33.56	C22H42O2	338.6	8216	Antimicrobial, antioxidant and hypocholesterolemic (Nalawade et al., 2015).
11	Hexadecanoic acid	19.162	1.58	C16H32O2	256.42	985	Antimicrobial, antioxidant, antifungal, solvent, hypocholesterolemic, emollient, anti-inflammatory, pharmaceutical agent and 5-Alpha reductase inhibitor (Petronzi et al., 2013).
12	Farnesol	19.971	13.48	C15H26O	222.37	445070	Anticancer, anti-inflammatory and immunosuppressant activities (Jung et al., 2018).
13	Squalene	26.502	7.69	C30H50	410.7	638072	Antimicrobials, antitumor agent, supplements, antioxidant, anti-cancer, repellents and hypocholesterolemic (Lozano-Grande et al., 2018).
14	Pentadecanoic acid	26.5	4.09	C15H30O2	242.4	13849	Antioxidant, antifungal and antimicrobial activities (Oni et al., 2020).

were almost comparable in both samples. Both extracts revealed the presence of flavonoids and alkaloids, which showed better inhibition against Shigella species, Salmonella species, Staphylococcus aureus and Escherichia coli. This research has revealed the antibacterial activity of these plant species, which confirms their effectiveness in traditional medicine and specifies their prospects in the development of modern drugs to fight microorganisms. Further research can be done on the plant with a reagent to isolate pure compounds from the plant, characterise them, and establish the exact phytoconstituents responsible for the antibacterial effect of *Thevetia peruviana* leaves.

CONFLICTS OF INTEREST

There are no conflicts of interest to disclose.

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AUTHOR CONTRIBUTIONS

Great Iruoghene Edo- Research concept and design, Collection and/or assembly of data, Data analysis and interpretation, Writing the article, Critical revision of the article and Final approval of the article.

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