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Research Article

Bioactive Components Identification and a Pilot Health Properties Study of *Tribulus terrestris L.*: A Native Saudi Arabian Plant

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Abstract

There are multiple wild plants in Saudi Arabia with unique phytochemical components that are reflected by the geographical desert such as *Tribulus terrestris Linn* from the *Zygophyllaceae* family with yellow flowers. The wonderful yellow flower is used to treat infertility, libido, and menopausal disorder as a traditional native medicine. This study aims to investigate the profile bioactive content and the antioxidant, cytotoxicity, and antimicrobial properties of *Tribulus terrestris L.* We studied the phytochemical profiling and antioxidant, cytotoxicity, and antimicrobial properties of 2 leaf extracts made from *Tribulus terrestris L.*, a native plant from Saudi Arabia. We analyzed the main phenolics using HPLC-DAD. Furthermore, we use GC/MS and GC-FID to identify the hydrocarbon profile in the study of non-polar extract. We studied the polar extract antioxidant activity using DPPH, ABTS, and Fe^{+2} chelation assays. We also assessed the activity as cytotoxicity and antibacterial. These findings highlight *Tribulus terrestris L.* as a valuable source of antioxidants with potential applications in food and medicine. We further explore bioactive components against cancer cells of HepG-2, MCF-7, and HeLa. Additionally, we investigated the antibacterial effect against bacteria: *S. aureus*. We report that *Tribulus terrestris L.* is a valuable source of antioxidants with potential cytotoxicity, and antimicrobial applications in food and medicine. Its bioactive components make it a promising candidate for further exploration.

Keywords: *Tribulus terrestris Linn*; GC/MS; HPLC-DAD; Antioxidant; Cytotoxicity; Antimicrobial; Medicinal plants

Introduction

Natural products are beneficial for medicinal sources and rich in bioactive constituents that have an excellent chance for cure benefits. Plant bioactive give forcefully advantageous constituents for use in food, health, and Medicinals [1]. Currently, the most bioactive selection that are serve to inhibit and relieve a wide range of health complications, including cancer and microbial infection, are phenolics and flavonoids [2-4]. The essential source of bioactive phenolics and flavonoids is medicinal roots, particularly those that flourish in thirsty regions with tough climatic circumstances. Due to its unique geographic position, Saudi Arabia's desert isn't the same as those grown in other regions or climates in terms of chemo diversity or medicinal fractions [5]. Saudi Arabia's herbs and flowers have a wide range of significant crops and remedial sources that are historically used for medicinal purposes, specifically in the traditional drug used by a major section of Saudi and non-Saudi people [6]. *Tribulus terrestris L.* is one of the most important sections of the family. *Zygophyllaceae* grows annually as a lying down growing spices with complex paripinnate leaves and unheroic 5 petals' flowers. Although it's primeval to the Mediterranean region, it presently distributes around the world in thirsty and semi-arid regions, including those in North Australia, Africa, Asia, South Europe, and America [7]. In Saudi Arabia, it grows wild across the country, especially in the Eastern Najd and Southern Hejaz areas, and generally known as "Darisa or Tikandu" [8]. Saudi Bedouin use *Tribulus terrestris L.* herbs to treat liver complications, sexual problems, energy deficiency, and urinary issues. Its seeds are specified for gout, restless leg, and hemorrhages, while its fruits are consumed as an aphrodisiac, diuretic, and erogenous [8]. Multiple studies including *in vitro* and *in vivo* experimental trails, as reported in the most recent review that demonstrated the anti-urolithic inflammation, general anti-inflammatory herb, anti-diabetic, anti-cancer, antibacterial, antispasmodic such as epilepsy, immunomodulatory, analgesic, cardiotonic, hypolipidemic, anthelmintic, hepatoprotective, diuretic, and larvicidal and fir intestinal worms [9]. *Tribulus terrestris L.* either, steroidal saponins fragments from *Tribulus terrestris L.* are set up in numerous pharmaceutical conventions that are listed as encyclopedically to treat manly sexual runs and libido issues in both men and women [10,11]. *Tribulus terrestris L.* excerpts involved a wide range of bioactive ingredients belonging to multiple classes including saponins, flavonoids, alkaloids, cinnamic

acid amides, amides and lignanamides, quinic acid derivations, phytosterols, adipose acids and adipose acid esters, and another effector [12]. Alkaloids, flavonoids, and steroidal saponins are allowed to be the most vital factors included in *Tribulus terrestris L.* components due to their colorful natural capabilities [12]. Alkaloids contain tribulusterine, perlolyrine, harmane, and harmaline [13]. Tricloside, kaempferol, quercetin, and isorhamnetin, and their derivations are exemplifications of flavonoids [14]. Protodioscin, prototribestin, neoprototribestin, and terestrinins are exemplify of furostanol saponins, whereas dioscin, tribestin, diosgenin, and tribulosin are example components of spirostanol saponins [15-17]. Multitudinous investigations report a link between the important natural impacts of effectors bioactive, particularly flavonoids and phenolics, to their strong antioxidant capacities, which specifically target for cure many health issues [18,19]. Pervious investigators report that the phytochemical components of *Tribulus terrestris L.* work to fight cancer through balance and decrease amounts of Reactive Oxygen Species (ROS) in the cell combined with elevated efficacity of endogenous and exogenous antioxidants to manage oxidative stress, which can lead to multitudinous affections, including cancer, diabetes, cardiovascular complaint, and neurodegenerative conditions [20]. These correlations constitute a vital need for maintaining a balance between the relative volume of ROS and antioxidants in the cell. To save such a balance, cells induce endogenous antioxidant enzymes, which transfigure free revolutionaries into stable and less dangerous foundations. Elevated ROS plays a disgraceful part in cancer initiative effect, they induce oxidative changes in DNA bases alterations, mutation, beach front breakage, elevates suppressor gene repression, and proto- oncogene induction, which convert healthy cells into tumors cells growth [20]. Thus, one of the most important strategies in cancer curatives has been the use of strong antioxidants that lower oxidative stress, such as natural products herbs [21]. But because recent studies have shown that synthetic antioxidants like Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) have cytotoxic goods, it's thus vital to find safe and effective antioxidants from natural sources to maintain many health insurance [22-24]. Numerous studies have revealed different remedial benefits of *Tribulus terrestris L.* in colorful locales; nonetheless, information on the chemical composition and natural characteristics of this medicinal factory that grows wild in the Al-Leith area of Saudi Arabia is lacking. therefore, from this point of view, we will identify the constituents' profile and assess the antioxidant, cytotoxicity, and antibacterial impacts of *Tribulus terrestris L.*, which was collected in the Saudi Arabian Al-Leith region.

Materials and Methods

Chemicals

These chemicals were obtained from Sigma-Aldrich Co., (USA); DPPH, ABTS, Folin- Ciocalteau reagent, Gallic acid, Quercetin, BHT, Trolox, Ferrozine, and phenolics standards Compounds. The others' reagents and detergents were also informed in each analytical process according to the instrument used.

Plant collection

In May 2022, we collect *Tribulus terrestris L.* plant from the Al- Leith area of Saudi Arabia. Dr. Abdallah A. AL-Feel from the Department of Arid Land Agriculture, King Abdulaziz University did the laboratory identification and recognized the studied taxonomy of the plant. A testimonial instance (TT52022) was deposited at Umm Al-Qura University's Chemistry Department, Faculty of Applied lores, Al-Leith University College. We apply the plant material to drying the sun system before grinding it into a fine by using mortal and thinking in an electrical grinder for preparation the powder for the extraction process. The experiments steps in detail in this study are demonstrated in Figure 1.

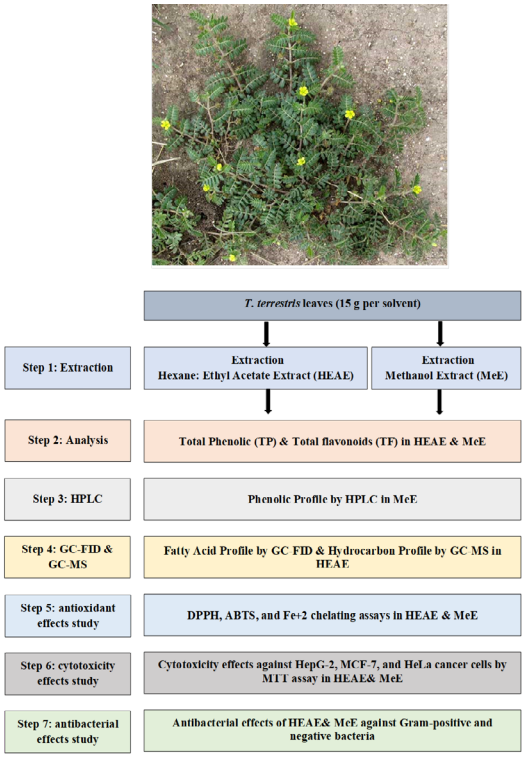

Figure 1: Experimental design

Plant extraction

We use Hexane: Ethyl acetate (2:1, v/v) and methanol (150 mL) to obtain 2 separate extracts. the grind *Tribulus terrestris L.* leaves (15 g per solvent) were used in each extract process. We used an orbital shaker (Stuart, England) for vortex the extraction solutions for 12 hours at room temperature at 160 rpm. We use Whatman No. 1 filter paper to filter the obtain extracts separately.

We wash the plant residues and 2 additional times using 150 mL of each fresh solvent for each one separately. We use rotary evaporator (Heidolph Unimax 2010, Germany) to concentrate the obtained filtrates separately. The yielding extracts were Hexane: Ethyl Acetate Extract (HEAE) and Methanol Extract (MeE) [18,19].

Phytochemical analysis

Estimation of Total Phenolics (TP): To determine the TP concentrations in HEAE and MeE, we combined 1 mL of the extract with 10 mL of distilled water and 1 mL of Folin reagent. We waited for 5 minutes, then we added 10 mL of 8% Na_2CO_3 to the mixture. We then added 8 mL of distilled water and vortexed the mixture for 15 seconds before putting them in dark incubation for 90 minutes at 25°C ± 2°C [19]. We determined the optical density of color, the measuring components spectrophotometrically at 750 nm as compared to a blank sample. We expressed the values of TP as mg Gallic Acid Equivalents (GAE)/g, D.W. We conducted 3 assessments for each sample [19].

Estimation of Total Flavonoids (TF): To determine the TF concentrations in HEAE and MeE, we combined 1 mL of the extract with 4 mL of distilled water and 0.3 mL of $NaNO_2$ (5%). After waiting for 5 min, we added 0.3 mL of 10% $AlCl_3$ to the mixture. After waiting for an extra 6 minutes, we added 2 mL of NaOH (1M) and 2.4 mL distilled water, then vortexed the mixture for 15 seconds before allowing it to stand for 10 minutes at 25°C ± 2°C [18]. We determined the absorbance of the yield mixture at 510 nm compared to a blank sample. We calculate the amount of TF as mg Quercetin Equivalents (QE)/g, D.W. We apply 3 measurements for each sample [18].

HPLC phenolic profile of MeE: We vortexed 10 mg of MeE in 2 mL methanol for 15 min., then filtrated it using A 0.2 μm Millipore membrane filter. We injected the filtrate (5 μL) into an HPLC (Agilent Technologies 1260 series, Germany) utilizing its auto-sampling injector. We isolated phenolic compounds at a constant temperature of 40°C ± 2°C and 0.9 mL/min flow rate using a 4.6 mm x 250 mm Eclipse C-18 column with a particle size of 5 μm. A mixture of water (A) and acetonitrile with 0.05% trifluoroacetic acid (B) was used as the mobile phase, with a linear gradient of 0 min (82% A), 0 min–5 min (80% A), 5 min-8 min (60% A), 8 min-12 min (60% A), 12 min-15 min (82% A), and 15 min-20 min (82% A). The DAD detector was checked at 280 nm [24]. The concentration of each specific phenolic component (mg/g of dry weight) is calculated by comparing its relative peak area with the reference standards such as Gallic acid, Chlorogenic acid, Catechin, Methyl gallate, Coffeic acid, Syringic acid, Pyro catechol, Rutin, and Ellagic acid from Sigma-Aldrich (Steinheim, Germany) [24,25].

Fatty acid and hydrocarbon analysis of HEAE

Fatty acid analysis by GC-FID: The former technique of Zahran and Tawfeuk was utilized to produce the Fatty Acid Methyl Esters (FAMEs) of HEAE. Hexane (1.0 mL) was added to 15 mg of the extract, and then 0.4 M sodium methoxide (1.0 mL) was added [26]. After 30 sec vortex, the mixture was allowed to settle for 15 minutes. The FAMEs were separated for gas chromatography (GC-FID) analysis from the upper phase. FAMEs were examined using a Perkin Elmer Auto System XL fitted with a ZB-Wax (capillary column, 60 mm x 0.32 mm) and a flame ionization detector (FID). We used helium as a carrier gas at one milliliter per minute flow rate. The oven was set to start at 50°C and increase in temperature by 4°C per minute. The injector and detector were adjusted to a temperature of 250°C [26].

Hydrocarbons analysis by GC/MS: The HEAE lipoidal matter was prepared by saponification, which involved refluxing 50 mg of the extract with 10% alcoholic potassium hydroxide for 5 hours in a water bath at 100°C ± 5°C. After evaporating the solvent, the precipitate was stirred with 50 mL of water. By using a separating funnel, the mixture was fractionated with ether (4 mL × 80 mL). The pooled ethereal fractions were cleaned from any alkalinity using distilled water, then dried up over Na_2SO_4 anhydrous. The solvent was concentrated using rotary, and the hydrocarbons (unsaponifiable substances) were analyzed using GC/MS spectroscopy [27]. The GC/MS comprises an Agilent 8890 gas chromatography equipped with an Agilent 5977B mass spectrometer and HP-5MS capillary column (30 m (L), 0.25 mm (ID), and 0.25 mm thickness). The temperature of the oven started at 50°C, then scheduled to increase by 5°C per minute until 280°C, and then held at that temperature for 25 minutes. We used helium as a carrier gas at one milliliter per minute flow rate. Using a split ratio of 1:50, hydrocarbons (1 μL) was injected automatically in the GC at 230°C. Mass spectra was scanned using the Electron Impact (EI) mode at 70 eV with scan range of m/z=39 amu-500 amu. The split peaks were detected by relating them to data from the NIST (National Institute of Standards and Technology) collection [27].

Antioxidant activity

DPPH• radical scavenging assay: After preparing 0.1 mM of DPPH• (2,2- diphenyl-1-picryl hydrazyl) in methyl alcohol, 1 mL of DPPH solution was added to 1 mL of HEAE and MeE at strengths of 25 μg/mL, 50 μg/mL, 75 μg/mL, and 100 μg/mL. After 15 sec vortex, the mixture was kept standing at 25°C ± 2°C in a dark area for 30 min. The readings were shown at 515 nm compared to a control sample [3]. All mixture components except for the extract were used in the control sample. The positive control was Butyl hydroxytoluene (BHT). We calculated the capacity to scavenge the DPPH• radical using this formula: Scavenging impact (%) of

$$\text{DPPH} = \frac{Ac - As}{Ac} X100$$

Where Ac: The control reading; As: The sample reading. The findings were expressed as IC_{50} (the sample's concentration in mg/mL that has a 50% DPPH• radical scavenging effect) [3].

ABTS•+ antioxidant assay: Using the 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) test, which was detailed by previous investigation [22]. The scavenging activity of HEAE and MeE was determined. Stalks of ABTS (7.4 mM) and potassium persulphate (2.6 mM) were freshly prepared using distilled water. The 2

stock solutions were then combined in identical parts to create the functioning solution. They were left to react at 25°C ± 2°C in a dark area for a duration of 12 hours to 16 hours. Then we mixed the functioning solution (1 mL) with methanol (60 mL) to make the ABTS solution, with a reading of 1.1 ± 0.02 at 734 nm by spectrophotometer. 150 μL of HEAE/MeE at concentrations of 250 μg/mL, 500 μg/mL, 750 μg/mL, and 1000 μg/mL was mixed with the ABTS solution (2850 μL), then kept standing for 2 hours at 25°C ± 2°C in the dark. The readings were shown at 734 nm compared to a control sample. All mixture components except for the extract were used in the control sample. The positive control was Trolox. We calculated the capacity to scavenge the ABTS radical using this formula:

$$\text{Scavenging impact (\%) of ABTS} = \frac{Ac - As}{Ac} X100$$

Where Ac: The control reading; As: The sample reading. The findings were expressed as IC_{50} (the sample's concentration in mg/mL that has a 50% ABTS radical scavenging effect) [22].

Ferrous ion (Fe^{2+}) chelating assay: Three milliliters of HEAE/MeE at various concentrations (0.75 mg/mL, 1 mg/mL, 1.25 mg/mL, 1.5 mg/mL) were mixed with 60 μl of 2 mM $FeSO_4$. To initiate the reaction, 100 μl of 5 mM ferrozine was added, the mixture mixed, and maintained standing for 10 min at 25°C ± 2°C. Using spectrophotometry, the mixture readings were calculated at 562 nm compared to a control sample [28]. All mixture components except for the extract were used in the control sample. The positive control was EDTA. We calculated the capacity to inhibit ferrozine-Fe^{2+} complex formation using this formula:

$$\text{Chelating activity (\%)} = \frac{Ac - As}{Ac} X100$$

Where Ac: The control reading; As: The sample reading. The findings were expressed as IC_{50} (the sample's concentration in mg/mL that inhibits 50% of ferrozine-Fe^{2+} complex formation) [28].

Cytotoxicity activity

Three human carcinoma cells: HepG-2 (hepatocellular carcinoma), MCF-7 (breast carcinoma), and HeLa (cervical carcinoma) were bought from the American Type Culture Collection (ATCC, Rockville, MD) [23].

Cell line propagation: The cells were propagated on Dulbecco's Modified Eagle's Medium (DMEM) involving gentamycin (50 μg/mL) and inactivated fetal bovine serum (10%). The cells were persistent by sub-culturing up to 3 times weekly and kept at 37°C in an environment with 5% CO_2 [23].

MTT cytotoxicity assay: In Corning® 96-well tissue culture plates, the tested cell lines (5 x 104 cells/well) were suspended in the medium, and the plates were incubated for 24 hours. After aspirating the media, new medium with varying doses (0.25 μg/mL-500 μg/mL) of dissolved HEAE and MeE in DMSO was applied to the cells. Each plate included 6 well controls, each of which was run with media or DMSO. The positive control was vinblastine sulfate (PubChem CID 5388983) as a standard chemotherapy cytotoxic agent against cancer cell as vinblastine sulfate blook cell division. The MTT test was used to count the live cells after a 24-hour incubation period, in accordance with the procedure outlined by previous investigation [23]. In brief, we removed the media from the plates and added 100 μl of fresh, phenol red-free medium. We added 10 microliters of 12 mM MTT (in PBS) to all the wells, including the control wells, and incubated the plates for 4 hours at 37°C ± 2°C with 5% CO_2. After that, we removed 85 μl of medium from each well and added 50 μl of DMSO. We mixed the solution using a pipette and incubated the plates at 37°C for 10 minutes. We estimated the number of *via*ble cells by measuring the absorbance readings of the wells at 590 nm with a microplate reader [23].

We calculated the viability (%) using this formula:

$$\frac{ODc - ODs}{ODc} X100$$

where ODc is the control absorbance reading and ODs is the sample absorbance reading. The results were expressed in terms of IC_{50}, which is the concentration (in μg/mL) of the extract that prevents 50% of contact cancer cells from growing [23].

Antimicrobial activity

In this study, the following germs were tested: *Staphylococcus aureus* (ATCC-47077) and *Bacillus cereus* (ATCC-12228) an example of Gram-positive bacteria, *Escherichia coli* (ATCC-25955) and *Proteus vulgaris* (ATCC-13315) an example of Gram-negative bacteria, filamentous fungus *Aspergillus niger* (ATCC- 16888), and yeast specie of *Candida albicans* (ATCC-10231). HEAE and MeE's antimicrobial activity was evaluated using a modified agar well diffusion technique [29]. In summary, 10 milliliters of fresh medium were cultured with 100 microliters of the test bacteria or fungus till 108 cells/mL of bacteria and 105 cells/mL of fungus are reached. Then we smeared 100 microliters of the bacterial culture onto nutrient agar plates to test their susceptibility using the well diffusion method. We hit holes with a diameter of 6 millimeters in the agarose gel and added 100 μl of the extract at doses of 2.5 mg/mL, 5 mg/mL, and 10 mg/mL to the well. The Petri dishes were incubated for 24 hours-48 hours at 37°C for bacteria and yeast and for 48 hours at 28°C for filamentous mold fungi. We measured the inhibition zones in millimeters (mm) as a scale to determine the efficiency of the antimicrobial effect. Since DMSO exhibited no inhibitory zone against any of the examined microbes, it was utilized as a negative control and to dissolve the tested extracts. Additionally, positive controls were performed using conventional medications such as gentamycin, an example of an antibacterial drug, and ketoconazole, an example of an antifungal agent [29].

Statistical analysis

Every test was run in triplicate. The means ± Standard Deviation (SD) of the data is presented. Using the SPSS,

software program (version No. 20), and one-way ANOVA, the values were expressed as the mean ± SD at the 0.05 significance level.

Results

Phytochemical analysis

In this work, we extracted bioactive components from *Tribulus terrestris L.* leaves using 2 distinct solvents: Hexane/ethyl acetate (2:1, v/v) and methanol. The polar solvent, methanol, had a maximum extraction yield of 11.63%, while hexane/ethyl acetate yielded 1.77%. The Methanol Extract (MeE) of *Tribulus terrestris Linn* possessed the greatest quantities of total phenolics (7.12 mg GAE/g ± 0.09 mg GAE/g, d.w) and total flavonoids (4.62 mg QE/g, d.w ± 0.08 mg QE/g, d.w), compared to 0.31 ± 0.003 (mg GAE/g, d.w) of total phenolics and 0.1 ± 0.002 (mg QE/g, d.w) of total flavonoids in the Hexane/Ethyl Acetate Extract (HEAE) as presented in Figure 2.

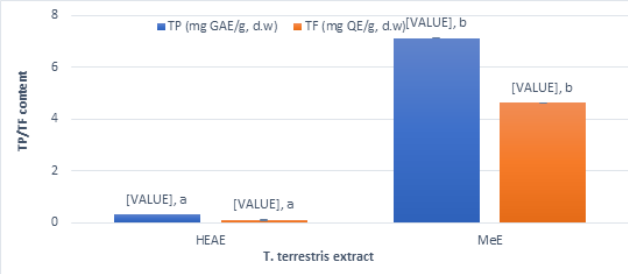

Figure 2: Total phenolics (TP, mg GAE/g, d.w) and total flavonoids (TF, mg QE/g, d.w) of Hexane/Ethyl Acetate Extract (HEAE) and Methanol Extract (MeE) of *Tribulus terrestris L.* Data are expressed as Mean ± SD (n=3). Different letters for the same parameter are significantly different at $p<0.05$

The individual phenolic and flavonoid components of the MeE were evaluated by HPLC-DAD. A total of 90 phenolic compounds were found in the MeE (Table 1). The main ones include the flavonoids hesperetin (2321.29 μg/g, d.w), quercetin (1770.84 μg/g, d.w), and daidzein (686.23 μg/g, d.w), and the phenolic acids chlorogenic acid (323.50 μg/g, d.w), cinnamic acid (230.94 μg/g, d.w), and gallic acid (127.72 μg/g, d.w), Figure S1 shows HPLC chromatogram of *Tribulus terrestris L.* methanol extract, signals at 280 nm (Figure 3).

Table 1: HPLC-DAD phenolic profile of *Tribulus terrestris L.* Methanol Extract (MeE)

No.	Compound	Concentration (μg/g, d.w)	Retention Time (min.)
Phenolic acids			
1	Chlorogenic acid	323.5	4.227
2	Cinnamic acid	230.94	14.027
3	Gallic acid	127.72	3.373
4	Ellagic acid	92.99	8.805
5	Coffeic acid	55.73	6.044
6	Syringic acid	37.79	6.576
7	Ferulic acid	32.3	10.239
8	Coumaric acid	12.28	9.135
Flavonoids			
9	Hesperetin	2321.29	15.517
10	Quercetin	1770.84	12.712
11	Daidzein	686.23	12.245
12	Naringenin	162.43	10.511
13	Catechin	153.17	4.609
14	Kaempferol	131.64	14.932
15	Apigenin	74.93	14.436
16	Rutin	30.86	7.988
Other phenolics			
17	Vanillin	92.53	9.754
18	Methyl gallate	18.22	5.592
19	Pyro catechol	4.05	6.76

The fatty acid composition and hydrocarbons composition of HEAE was detected and presented in Table 2. Six fatty acids in total were detected using GC-FID, and the main fatty acids found in HEAE were palmitic acid (C16:0), linoleic acid (C18:2n6c), and oleic acid (C18:1n9c) in quantities of 19.25%, 28.52%, and 35.82%, respectively. Five hydrocarbons were detected in HEAE using GC-MS analysis, and the most abundant of which is squalene (39.5%), phytol (28.56%), and tetracosanol (19.99%), Figure 4 shows GC/MS chromatogram of *Tribulus terrestris L.* hexane/ethyl acetate extract hydrocarbons.

Table 2: Fatty acid composition (%) and hydrocarbons composition (%) of Tribulus terrestris L. Hexane/Ethyl Acetate Extract (HEAE)

No.	Fatty acid	fatty acids (%)	Hydrocarbon	Hydrocarbon (%)
1	Palmitic acid (C16:0)	19.25	Phytol	28.56
2	Stearic acid (C18:0)	0.76	Hexanedioic acid, bis(2-ethylhexyl) ester	2.91
3	Oleic acid (C18:1n9c)	35.82	Docosanol	6.13
4	Linoleic acid (C18:2n6c)	28.52	Tetracosanol	19.99
5	α-Linolenic acid (C18:3n3)	4.71	Squalene	39.5
6	Arachidic acid (C20:0)	10.94	-	-

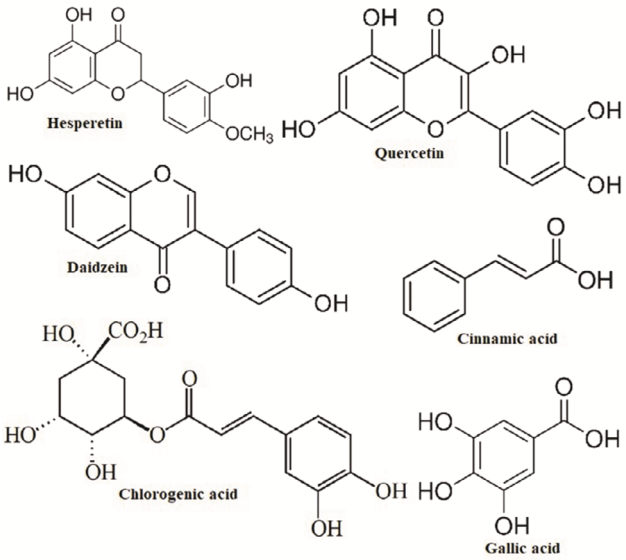

Figure 3: The chemical structures of the main analytes were identified using the HPLC-DAD technique

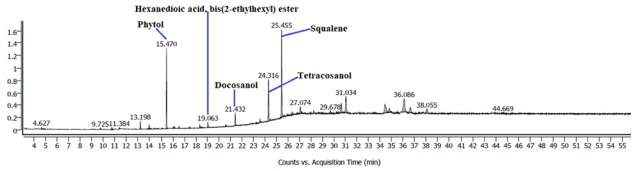

Figure 4: GC/MS chromatogram of *Tribulus terrestris Linn* hexane/ethyl acetate extract hydrocarbons

Antioxidant activity

Using 3 different assays (DPPH•, ABTS•+, and Fe^{+2} chelation), we assessed the *Tribulus terrestris L.* extract's *in vitro* antioxidant activity. The findings were reported as IC_{50} values. Since the IC_{50} value and antioxidant activity are inversely correlated, analyte antioxidant activity increases with decreasing IC_{50} value. The MeE exhibited the greatest antioxidant capacity in all 3 experiments, with IC_{50} DPPH 0.138 mg/mL, IC_{50} ABTS 0.287 mg/mL, and IC_{50} Fe^{+2} chelation 1.293 mg/mL. In comparison, the HEAE had IC_{50} DPPH 0.174 mg/mL, IC_{50} ABTS 0.721 mg/mL, and IC_{50} Fe^{+2} chelation 1.525 mg/mL. Figure 5 show individual (DPPH•, ABTS•+, and Fe^{+2} chelation) scavenging activity (%) of Hexane/Ethyl Acetate (H/E) and Methanol (Me) extracts of *Tribulus terrestris L.* and Methanol (Me) extracts of *Tribulus terrestris L.*

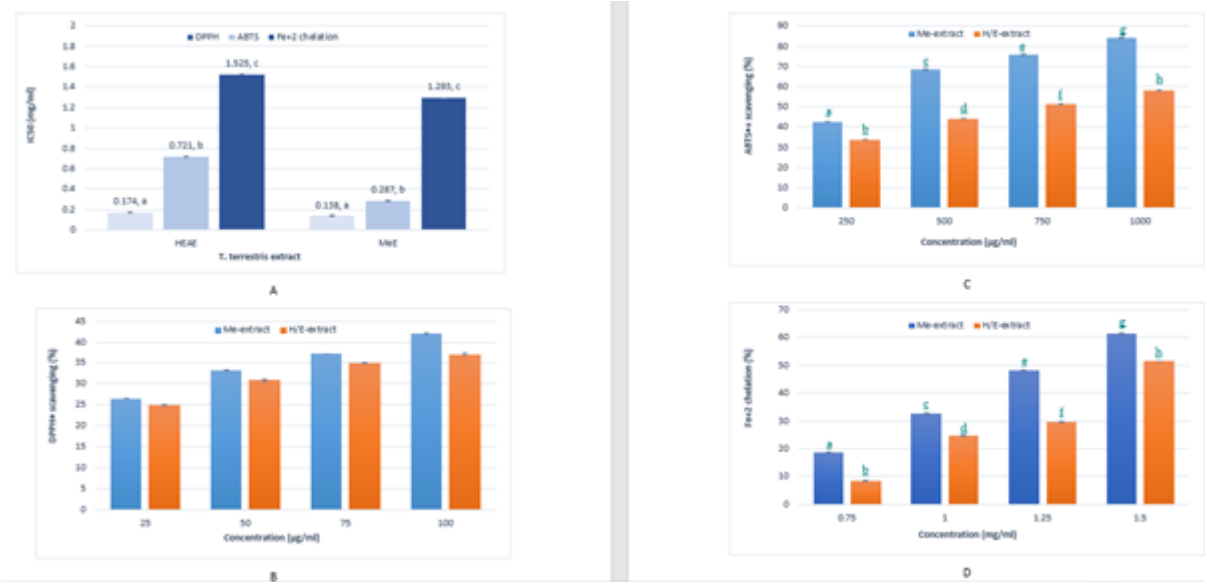

Figure 5: A) Antioxidant activities (IC50, mg/mL), DPPH scavenging, ABTS scavenging, and Fe^{+2} chelation effects, of Hexane/Ethyl Acetate Extract (HEAE) and Methanol Extract (MeE) of Tribulus terrestris L. Data are expressed as Mean ± SD (n=3). Different letters for the same parameter are significantly different at $p<0.05$; B) DPPH• scavenging activity (%) of Hexane/Ethyl Acetate (H/E) and Methanol (Me) extracts of Tribulus terrestris L. Data are expressed as Mean ± SD (n=3). Different letters for each extract (MeE and HEAE) individually are significantly different at $p<0.05$; C) ABTS•+ scavenging activity (%) of Hexane/Ethyl Acetate (H/E) and Methanol (Me) extracts of Tribulus terrestris L. Data are expressed as Mean ± SD (n=3). Different letters for each extract (MeE and HEAE) individually are significantly different at $p<0.05$; D) Fe^{+2} chelation activity (%) of Hexane/Ethyl Acetate (H/E) and Methanol (Me) extracts of Tribulus terrestris L. Data are expressed as Mean ± SD (n=3). Different letters for each extract (MeE and HEAE) individually are significantly different at $p<0.05$

Cytotoxicity activity

Using MTT assay, we assessed the *Tribulus terrestris L.* extract's *in vitro* cytotoxicity efficiency against HepG-2, MCF-7, and HeLa cancer cells as compared with Vinblastine sulfate as a standard. The findings were reported as IC_{50} values. Since the IC_{50} value and cytotoxic effect are inversely correlated, analyte cytotoxic effect increases with decreasing IC_{50} value. The HEAE exhibited the most potent cytotoxicity against the HepG-2, MCF-7, and HeLa cell lines, with IC_{50} values of 46.71 ± 1.43, 61.38 ± 1.97, and 75.41 ± 2.31 μg/mL, respectively. In comparison, the MeE showed IC_{50} values of 103.79 ± 3.08, 107.57 ± 3.29, and 157.52 μg/mL ± 5.83 μg/mL for the same cell lines (Figure 6). As shown by the lowest IC_{50} values, the HepG-2 cell line is the most sensitive to *Tribulus terrestris L.* extracts

followed by MCF-7, then HeLa.

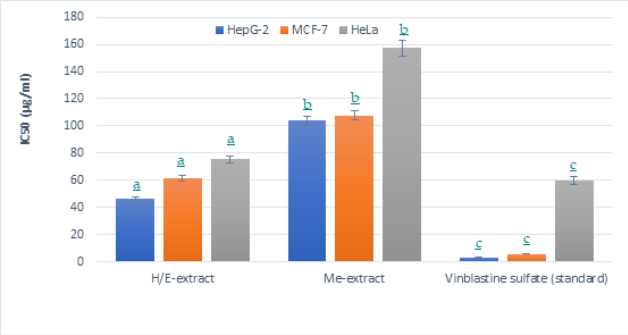

Figure 6: Cytotoxic effect (IC_{50}, μg/ml) of Hexane/Ethyl Acetate (H/E) and Methanol (Me) extracts of *Tribulus terrestris L.* against HepG-2, MCF-7, and HeLa cancer cell lines as compared to Vinblastine sulfate standard. Data are expressed as Mean ± SD (n=3). Different letters for each extract MeE and HEAE, Vinblastine sulfate individually are significantly different at $p<0.05$

Antimicrobial activity

We assessed the *in vitro* antimicrobial effectiveness of *Tribulus terrestris L.* HEAE and MeE versus several bacteria, including *S. aureus*, *B. cereus*, *E. coli*, and *P. vulgaris*, as well as the fungus *A. niger* and yeast *C. albicans*, by employing the agar well diffusion method. The results were expressed in terms of the inhibition zone (mm). In general, HEAE and MeE demonstrated modest bactericidal activities depending on concentration but had no influence on the growth of the fungus *A. niger* (Table 3). The maximum growth inhibition against *S. aureus*, *E. coli*, *P. vulgaris*, and *C. albicans* was demonstrated by the HEAE at 10 mg/mL, with inhibition zones of 7.5 mm ± 0.3 mm, 8.5 mm ± 0.4 mm, 12.8 mm ± 0.3 mm, and 6.5 mm ± 0.1 mm, respectively. *P. vulgaris*, a gram-negative bacterium, is the most sensitive microbe to *Tribulus terrestris L.* HEAE and MeE.

Table 3: Antimicrobial effect of Hexane/Ethyl Acetate Extract (HEAE) and Methanol Extract (MeE) of *Tribulus terrestris Linn*

Microbes	Inhibition zone (mm)						Positive control
	2.5 mg/mL		5 mg/mL		10 mg/mL		
	HEAE	MeE	HEAE	MeE	HEAE	MeE	
Gram-positive bacteria							
S. aureus	NI	NI	6.7 ± 0.1	NI	7.5 ± 0.3	6.8 ± 0.3	24.3 ± 0.6
B. cereus	NI	NI	6.5 ± 0.2	7 ± 0.4	7.3 ± 0.4	7.7 ± 0.2	26.3 ± 0.5
Gram-negative bacteria							
E. coli	NI	NI	6.6 ± 0.2	NI	8.5 ± 0.4	7.5 ± 0.2	29.6 ± 0.6
P. vulgaris	7.3 ± 0.2	6.5 ± 0.1	9.5 ± 0.4	8.2 ± 0.4	12.8 ± 0.3	11.5 ± 0.4	26.7 ± 0.6
Yeast							
C. albicans	NI	NI	NI	NI	6.5 ± 0.1	NI	21 ± 0.5
Fungi							

The diameter of the well (6mm) is included; NI: No inhibition zone; Gentamycin at 100 μg/mL used as positive control for antibacterial test; Ketoconazole at 500 μg/mL used as positive control for anti-yeast and antifungal tests

Discussion

Several herbs, wild and edible, have many different medical properties depending on their multiple range of bioactive components and benefits phytochemical. Medicinal plants are used global in traditional prescriptions worldwide in healing or preventing several human illnesses. Furthermore, the components, effects, and quantity of bioactive constituents that are separated and identified from medicinal plants are extremely dependent on the extraction and the solvents composition. Phenolic compounds and their glycosides, for example, are extract using polar solvents, whereas steroids and fatty acids are extracted using non-polar solvents [29,30]. In this study, the polar solvent, methanol, was found to have a maximum extraction yield of 11.63%, which is higher than that of *Tribulus terrestris L.*'s aqueous (10%) and methanolic (9.3%) extracts [31]. Numerous investigations have reported the effects of many solvents on the quantity, effect, and composition of bioactive recovered from several medicinal plants [19, 30]. The maximum extraction yield of highly polar solvents, such as methanol, reveals that the crude extracts contain a high quantity of phenolic substances [30, 32].

The findings of current work indicate that the Methanol Extract (MeE) of *Tribulus terrestris L.* contained higher amounts of total phenolics and total flavonoids than the Hexane/Ethyl Acetate Extract (HEAE). The current findings agree with previous results investigation, who also reported that the methanol and ethanol extracts of *Tribulus terrestris L.* includes the highest levels of total phenolics and total flavonoids compared to the hexane extract [33]. The adequate quantities of total phenolics and total flavonoids of *Tribulus terrestris L.* collected from the Saudi Arabian Al-Leith region in current study are higher than that of *Tribulus terrestris L.* collected from the Saudi Arabian Najran region [7] and are comparable with that of *Tribulus terrestris L.* collected from various other countries [14,22,31,33,34].

In current investigation, methanol is the most proper solvent for phenolic and flavonoid extraction from *Tribulus terrestris L.* Given that the extract of phenolic compounds varies based on their structural characteristics, the HPLC analysis output indicated the presence of flavonoids hesperetin, quercetin, and daidzein, as well as phenolic acids chlorogenic acid, cinnamic acid, and gallic acid as

the essential source phenolics in the MeE. These findings are comparable with recent studies that identified several flavonoids, phenolic compounds, and flavonoid glycosides in *Tribulus terrestris L.* from several geographical areas [14,22,35,36]. The geographic location, method of extraction, plant part utilized, and solvent utilized have a substantial impact on the concentrations of phenolics and flavonoids [14,22,30,32].

In the current study, we determine that hexane/ethyl acetate is the most suitable solvent for extracting fatty acids and hydrocarbons from *Tribulus terrestris L.* The key fatty acids identified in HEAE were palmitic acid (C16:0), linoleic acid (C18:2n6c), and oleic acid (C18:1n9c). The current results agree with those of previous finding, who demonstrated" that "the key fatty "acids detected in *Tribulus terrestris L.* collected from various areas in Turkey were linoleic acid, oleic acid, and palmitic acid [33]. In contrast to the previous finding who identified the principal fatty acids found in *Tribulus terrestris L.* as oleic, pentadecanoic acid, 9,12-octadecadienoic acid, 6,9,12,15-docosatetraenoic acid, and palmitic acid [37]. In the present experiment results, GC-MS analysis characters a group of beneficial contents such as squalene, phytol, and tetracosanol as the main hydrocarbons in the unsaponifiable fraction of HEAE of *Tribulus terrestris L.* Otherwise, previous investigations using GC-MS analysis have also revealed that *Tribulus terrestris L.* contains phytol and squalene, along with other hydrocarbons [7,38]. The variations seen in the fatty acid contents and hydrocarbon composition of *Tribulus terrestris L.* between this study and recent publications might potentially be referred to several factors such as the geographical climates, the extraction technique, method, utilized solvents, and the plant part. Research has shown that the chemical composition is closely related to the plant's place of origin and, consequently, to the climate, that diversity give the studied plant different benefits, anti-bacterial, anti-cancer, and anti-oxidant [15,29,39].

The endogenous human antioxidants system is powerful and unfortunate for the current inactive lifestyle and Western diet. It is crucial to admit that human body health well-being needs more exogenous antioxidants from diet such as vegetables, fruits, and herbs. Antioxidants have a powerful capacity to protect and prevent cellular oxidative stress, decline DNA mutation, and keep human Healthline on wealthy activities, particularly from disorders connected to oxidative stress [39]. Therefore, assessing *Tribulus terrestris L.* extracts' antioxidant capacities is considered to understand how effective the plant is in preventing and treating oxidative stress-related issues. The MeE showed the highest antioxidant capacity when tested using 3 different assays (DPPH•, ABTS•+, and Fe^{+2} chelation) in comparison to HEAE. This can be attributed to the significantly higher quantities of phenolic and flavonoid compounds present in the MeE as reported in the current results. These findings are agreed upon with the previous study of [33]. They revealed that the methanol and ethanol extracts of *Tribulus terrestris L.*, which contain high amounts of polyphenolics, showed stronger DPPH antioxidant properties in comparison to the hexane extract. There is genuine data demonstrating the significant relationship between plant extracts' phenolic and flavonoid amounts and their antioxidant efficiency [23,30,32]. Numerous studies have indicated that different extracts of *Tribulus terrestris L.* showed antioxidant capacities using many tests such as DPPH, reducing power, ABTS, and FRAP [14,22,33,35,36]. This study found that MeE and HEAE exhibited their antioxidant activity by 2 different mechanisms: Metal chelating, as shown by the Fe^{+2} chelation test, and electron or hydrogen atom transfer, as shown by the DPPH and ABTS analysis. The antioxidant influence of the extract is derived from the synergistic impact of the phytoconstituents, which is dependent on their concentration as well as their structure and interaction [23,32]. Because of their redox characteristics, phenolic compounds display antioxidant activity *via* a variety of potential pathways, including the decomposition of peroxides, neutralization and absorption of free radicals, transition of metal chelating activity, and/or hunting of singlet and triplet oxygen [39]. These findings suggest that *Tribulus terrestris L.* extracts, with their potent antioxidant activity, might be adequate choice for developing natural, safe, and healthful antioxidants for use in food preservers as heathy additive and pharmaceutical applications such as anti-inflammatory and anti-cancer [36,39,40].

Cancer is one of the most painful deadly causes of mortality worldwide. One of the cancer treatments is chemotherapy such as vinblastine sulfate, which has painful side effects on the healthy cell, tissue, and organ of the patient's body [40,41]. In spite of several trails for select treatment methods without side effects, there is still a need for more and selectivity problems with chemotherapy medications, despite enormous attempts to develop effective ones. Therefore, several searches for new innovative medicines for cancer cells but not to healthy cells are concerning many researchers. Now, researchers are currently searching for new, safe, and valuable cytotoxicity agents derived from natural medicinal herbs [40]. As a result, testing *Tribulus terrestris L.* extracts for cytotoxicity activities are important to determine how effective the plant is in inhibiting cancer proliferation. The HEAE of *Tribulus terrestris L.* in the current study exhibited the strongest cytotoxic effect against HepG-2, MCF-7, and HeLa cell lines, compared to MeE. The present findings are comparable with the previous results that reported the cytotoxicity effects of *Tribulus terrestris L.* different extracts against different cancer cells including HepG2 cells, MCF-7 cells, cell lines of A2780 (ovary), HT29 (colon), and MCF7, cell lines of NCI-ADR/RES (ovary), OVCAR-03 (ovary), U251 (glioma), 786-0 (kidney), PC-3 (prostate), NCI-H460 (lung), HT-29 (colon), and MCF7, and cell lines of SK-OV-3 (ovary), NCI-H522 (lung), HeLa, and MCF-7 [11,40,41,43]. The effects of HEAE in current study on MCF-7 and HeLa with IC_{50} values of 61.38 μg/mL, and 75.41 μg/mL, respectively, are comparatively higher than those of methanol and 70% aqueous methanol extracts, with IC_{50} values of 74.1 μg/mL and 176.4 μg/mL and 221.2 μg/mL and 343.1 μg/mL, respectively [44]. The HEAE that exhibited the highest

cytotoxic impact in current investigation was also the one with the lowest quantities of total phenolics, total flavonoids, and antioxidant effects. Therefore, the current outcomes reflect those bioactive substances other than phenolics, hydrocarbons of phytol and squalene or saponins, impact *Tribulus terrestris L.* cytotoxicity findings. In similar results previous report found an cytotoxicity effect of *Tribulus terrestris L.* saponin-enriched extract, attributing the action to the presence of gitogenin, protodioscin, and diosgenin saponins in the extract [43]. Previous researchers report that phytocomponents other than phenolics, such as stigmasterol and sitosterol, may be affected and result in the cytotoxicity action of *Tribulus terrestris L.* extract [11]. In addition, previous study reported that one of the phytocomponents affected the biological activity of *Tribulus terrestris L.* extract [44]. *Tribulus terrestris L.* extracts have anti-inflammatory, antioxidant, and cytotoxicity impacts, may be due to the saponins of protodioscin and physcion [44]. Furthermore, many investigators surveyed the cytotoxicity activities of phytol and squalene hydrocarbons that agree with current results [45,46].

The current study also investigates the effect of *Tribulus terrestris L.* extracts as anti-bacterial. Therefore, bacteria resistance and infection are widely elevated due to many factors such as increased antibiotics used, climate change, sedentary lifestyle, and diet, which poses a severe threat to global public health. Therefore, continuing search for new innovative medicines as antibacterial is concern medical society. Herbs may yield a new, powerful source of antimicrobial activity, and a demand focus to phytochemicals and separate the biologically active components are commonly used in ancient and recent herbal medicine [29,47]. So, current work concerns reveal a pilot survey to study the effectiveness of the plant in preventing infectious disorders of *Tribulus terrestris L.* extracts through tested for antibacterial activity of several individuals' microbes. In the current study, HEAE and MeE of *Tribulus terrestris L.* demonstrated modest bactericidal activities depending on concentration but had no influence on the growth of the fungus *A. niger*. The HEAE exhibited the maximum growth inhibition against *S. aureus*, *E. coli*, *P. vulgaris*, and *C. albicans* at 10 mg/mL, with inhibition zones of 7.5 ± 0.3, 8.5 ± 0.4, 12.8 ± 0.3, and 6.5 ± 0.1 mm, respectively. According to previous report that the methanol extracts of *Tribulus terrestris L.* fruits and leaves showed effective antibacterial and antifungal activity against the tested microbes [34]. Their impact against *S. aureus* was higher than the current HEAE and MeE results, with diminish zones of 17 mm ± 1.1 mm and 18 mm ± 0.86 mm, respectively. With inhibition zones of 19.23 mm and 14.04 mm and 19.88 mm and 14.60 mm, respectively, other study also reported that methanol and aqueous extracts of *Tribulus terrestris L.* leaves presented significant antibacterial effect against *S. aureus* and *E. coli* [31]. These impacts were superior to the current HEAE and MeE results. The antibacterial influences of *Tribulus terrestris L.* HEAE in this study may be because of bioactive other than phenolics, such as fatty acids or hydrocarbons like phytol and squalene, the main contents were identified in the HEAE. These findings disagree with previous studies, they attributed the antibacterial effects of *Tribulus terrestris L.* to the presence of phenolics and flavonoids only [14,34]. Several investigations have been conducted on the antibacterial qualities of phytol and squalene, which provide a confirmation to our theory regarding their implications in the antibacterial activity of HEAE [46,48]. Furthermore, there are many of data that points to the possibility concern study plant bioactives have the capacity to interrupt the construction of the bacterial cytomembrane, elevates its permeability, fluidity, membrane protein delocalization, and other antibacterial activity-related activities [49]. According to the current findings, *Tribulus terrestris L.* leaf extracts may be acceptable natural medication to replace or reduce the usage of antibiotics in bacterial illnesses.

In summary, *Tribulus terrestris L.* is considered as a member of *Zygophyllaceae*. The ancient plant is used in many traditional medicinal cultures such as Saudi Arabia, Chinese, Indian, and European traditions for many health benefits. Different studied extracts of T. terrestris reflect a content of antioxidants phytochemical components with anti-inflammatory activities, cytotoxicity, and antimicrobial that agree with current results [40-49]. *Tribulus terrestris L.* consider as great promise as a source of antioxidants with applications in both food and medicine. Its bioactive constituents make it an exciting candidate herb for further research.

Conclusion

The author concluded that the effect of primerary data *Tribulus terrestris L.* from the Al-Leith area of Saudi Arabia as the antioxidant, cytotoxicity, antimicrobial due to phytochemicals components. The author confirm presence of bioactive phenolic of *Tribulus terrestris L.* such as hesperetin, quercetin, daidzein, chlorogenic acid, and cinnamic acid, among the ninety phenolics in the Methanol Extract (MeE). In addition, the study reports the presence of hydrocarbons such as phytol and squalene, the fatty acids linoleic acid, and oleic acid in the Hexane/ Ethyl Acetate Extract (HEAE). We also assessed MeE antioxidant activity, as demonstrated by DPPH, ABTS, and Fe^{+2} chelation and reported a significant antioxidant activity. The author concluded that HEAE had the strongest cytotoxicity activity against cancer cells HepG-2, MCF-7, and HeLa. In addition, the HEAE had the strongest antibacterial effectiveness versus *S. aureus*, *B. cereus*, *E. coli*, P. vulgaris, and *C. albicans*. These findings suggest that *Tribulus terrestris L.* extracts as an excellent option for establishing natural, safe, and effective alternatives for study and use in food and pharmaceutical applications because of their strong antioxidant, cytotoxicity, and antibacterial impacts, that need further exploration in progress in author next investigation steps.

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Conflict of Interest

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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