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HEADSPACE GC/MS AND LC/MS ANALYSIS OF BIOACTIVE COMPOUNDS FROM *GOSSYPIUM BARBADENSE* L. STEM AND ASSESSMENT OF THEIR ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES

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ABSTRACT. In line with the global interest and demand in finding sustainable ways to the emerging antimicrobial and cytotoxic activities, our study highlights the unique chemical composition of *G. barbadense* stem. Petroleum ether extracts were analyzed via headspace-gas chromatography/mass spectrometry (HS-GC/MS) revealing 48 compounds, thirteen major and accounting for 56.29% of total peak areas. By using liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) identify 16 major phenolic compounds from the methanolic extract. Both petroleum ether and methanolic extracts were tested for antibacterial activity against four pathogenic bacterial strains (*Escherichia coli* ATCC10536, *Staphylococcus aureus* ATCC6538, *Salmonella enterica* ATCC 14028, and *Pseudomonas aeruginosa* ATCC 9027) by using disk diffusion method. The antifungal activity against two fungal strains (*Aspergillus niger and Aspergillus fumigatus)* was evaluated. The results were compared to the activity of ciprofloxacin as a stander. The *in-vitro* cytotoxic activity of the methanolic extract showed a potent effect against liver (HEPG2) with IC_{50} values (18.8±7.4 µg/mL) and moderate activity against colon (HCT116) and breast (MCF7) cancer.

KEY WORDS: *Gossypium Barbadense* L. Stem, Headspace/GC/MS, LC-ESI-MS, Bioactive compounds, Antimicrobial, Cytotoxic activities

INTRODUCTION

Gossypium barbadense is a new world cotton plant that was introduced into indigenous medicine systems. This plant has been used to make medicines. It is a major commercial crop that is also known as white gold. *G.barbadense* is found throughout the tropics and subtropics. Which was used by ancient people in folk medicine to treat bronchial asthma and was used in the preparation of medicines to treat skin diseases and infections. Plant flowers are galactagogues and can be used to make herbal medicines to increase lactation and breast milk production in new mothers. Plant leaves are used as a first-aid remedy to stop bleeding in cuts, bruises and wounds. This plant's phytochemistry reveals the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins and terpenoids. This plant has anti-bacterial, anti-convulsant, anti-depressant, anti-diabetic, anti-fertility, anthelminthic, antioxidant, anti-poisonous, anti-spermatogenic, antitumor, anti-ulcerand anti-viral properties. It is used as an abortifacient, a contraceptive and a diuretic [1]. Therefore, this report aims to highlight its phytochemical and pharmacological study.

The plant has the capacity to be a source of vital compounds such as terpenes, phenolics, fatty acids, lipids, carbohydrates and proteins [2]. These compounds, which are found in the plant's seeds, bolls, calyx, leaves, stalks, stems and roots, serve important biological functions in humans and animals [3]. *G. barbadense* contains chemicals that may be useful to humans and animals, such as gossypol, a polyphenolic with possible contraceptive effects [4, 5] and trans-

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caryophyllene, a terpenoid with anti-inflammatory and cytotoxic activities [6, 7]. As a result of the spread of chemical compounds throughout the *G. barbadense* plant, by-products generated by the *G. barbadense* industry may provide a potential source of valuable extractives.

G. barbadense derived chemicals elicit a variety of responses in most experimental cells and animals, according to in *vitro* and in *vivo* investigations. Monoterpenes found in *G. barbadense* leaves, such as pinene, have antimicrobial effect against fungus and bacteria. Anti-microbial action against bacteria and fungi has been reported at concentrations as low as 5 µg/mL and 117 µg/mL, respectively [8, 9]. This impact was only seen in the compound's positive enantiomers. Another component found in cotton leaves is phenolic acid, which has antimicrobial effects against both gram positive and gram-negative bacteria with an IC_{50} value of 160 µg/mL [10]. The antimicrobial action of these chemicals varies depending on the microorganism. This was observed in phenolic acid-induced fungal toxicity tests on *Ganoderma boninense* at doses as low as 0.5-2.5 µg/mL [11].

It is estimated that more than 60% of anticancer medications currently in use are derived in some way from plants and other natural products and are being evaluated [12]. A successful anticancer treatment should destroy cancer cells while inflicting minimal harm to normally dividing cells. This concept is difficult, if not impossible, to grasp, which is why cancer patients frequently experience unpleasant side effects when undergoing therapy [13]. It has been stated that the seeds and roots of *G. barbadense* are used to treat nasal polyps, uterine fibroids as an abortifacient, and other types of cancer [14]. Gossypol (a toxic dihydroxy phenol) found in *G. hirsutum* seeds has anticancer action in novel LL, WA, and PS-150 tumor systems [15]. In Iranian folk medicine, the capsules of the plant known as "stems" were used to cure cutaneous leishmaniasis (Cl) [16]. This is accomplished by utilizing critical preliminary data supplied by cytotoxicity screening models [17]. The majority of tissues require oxygen to function. Oxygen-derived species generate free radicals, which cause chain reactions and attach to macromolecules, most commonly proteins, DNA, and lipids, resulting in chronic degenerative diseases such as cancer, diabetes, cardiovascular, and neurologic problems [18]

As part of ongoing attempts to fully use the therapeutic characteristics of natural products, the antibacterial and antifungal activity of petroleum ether and methanolic extracts of dried fresh powdered stems of *G. barbadense* were investigated in this study. In addition to exploration the in *vitro* cytotoxic activity of methanolic extract of *G. barbadense* stem towards human colon (HCT116), liver (HEPG2) and breast (MCF7) cell lines using the MTT assay.

EXPERIMENTAL

Plant collection

G. barbadense stem was gathered in September 2021 from a natural field in Zagazig - Sharqia, Egypt. It was identified by Prof. Dr. Alaaeldin Sayed Ewase, Ministry of Environment; Nature Conservation Sector, Biodiversity Administration, Cairo, Egypt. A voucher herbarium specimen (No. M145) was deposited in the National Research Center's (NRC) herbarium in Giza, Egypt, with the global code (CAIRC).

Extraction

G. barbadense stem was washed multiple times with double distilled water and air dried at room temperature. Stem fragments were carefully grounded into a powder (50 g), and defatted using the Soxhlet equipment, with petroleum ether at 60-80 ºC, followed by methanol. The crude two extracts were concentrated till dryness using Rotavapor® (Heizbad Hei-VAP, Heidolph, Germany). Yielding 16.58, 13.91 g, respectively, were stored at -4 ºC for further investigations.

Phytochemical screening

The methanolic extract was produced in two-dimensional paper chromatography in BAW and sprayed with ($FeCl_3$) after drying; some spots turned dark blue, indicating the presence of phenolics and flavanoids compounds [19].

Identification of essential oil components by headspace GC/MS

Three grams of air-dried and crushed sample were placed into a 20 mL headspace vial and immediately sealed with silicone rubber septa and aluminum caps for the absorption of the volatile compounds. They were transferred to the headspace and heated up to 80 °C for 20 min while being agitated, and then introduced directly into the GC injector with a loop temperature of 120 °C, and transfer line temperature of 140 °C.

Headspace GC/Mass Spectrometry is an Agilent Technologies system (7890B), equipped with a mass spectrometer detector (5977A), headspace sampler (7697A), and HP-5MS capillary column (30 m x 0.25 mm). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 1.0 mL/min with a spitless mode and the following temperature program: 50 °C for 2 min; rising at 10 °C /min to 250 °C; rising at 15 °C/min to 300 °C and held for 10 min. The injector and detector were held at 280 °C and 300 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 30-550 and solvent delay 5 min. [20]. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS)

The analysis was carried out at Drug Discovery and Development, Research Centre (Ain Shams University, Giza, Egypt) that used an inverse stage C_{18} column to isolate phenolic acids and flavonoids of methanolic extract. The sample solution (100 µg/mL) was prepared with MeOH HPLC grade solvent, and analyzed using LC-ESI-MS. The sample amounts (10 µL) were injected into the LC-ESI-MS apparatus. Gradient mobile phase elution was carried out at a flow rate of 0.2 mL/min using two eluents: H_2O acidified with 0.05% formic acid and acetonitrile. In negative ion mode, the following parameters were used: source temperature 150 °C, desolation temperature 440 °C, mass spectra were identified using the ESI negative ion mode. Using the Maslynx 4.1 software peaks and spectra were evaluated and tentatively identified by comparing retention time (R_t) with mass range and international data.

General

All chemicals used were the highest quality and analytical grade and purchased from Sigma-Aldrich and Merck (Germany).

Antimicrobial activity

The antimicrobial activity of petroleum ether and methanolic extracts was evaluated using the disc diffusion method [21]. The bacterial strains used were (*Escherichia coli* (*E. coli*) ATCC10536, *Staphylococcus aureus (S. aureus)* ATCC6538, *Salmonella enterica* (*S. enterica*) ATCC 14028 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 9027, that were preserved as a pure culture at -80 °C at the laboratory, Faculty of Science, Zagazig University, Egypt. The two extracts were dissolved in DMSO, and a solution of 1 mg/mL concentration was produced. The Müller-Hinton agar medium (MHA) for antibacterial activity (0.2 g/L beef extract, 17.5 g/L acid hydro lysate of casein, 1.5 g/L starch and 17 g/L agar) was prepared, autoclaved and then cooled

to 47 °C, and poured on sterilized petri dishes. After solidification, plates were streaked with the tested bacterial strains using sterile cotton swabs. After that, sterilized Whatman filter papers no. 1 with a diameter of 6 mm were impregnated with appropriated extract were placed on the agar surface plates using sterile forceps and gently pressed down to ensure complete contact with the agar surface. Then, the plates were incubated aerobically at 37 °C overnight. After that the diameter of each inhibition zone was measured with a ruler and recorded in mm.

The two fungus strains *Aspergillums niger* (*A. niger*) and *Aspergillums fumigatus* (*A. fumigatus*), which were isolated from Egyptian soil and water and then identified according to the standard mycological keys for identification of fungi were used for estimating the antifungal activity of petroleum ether and methanolic extracts. The Czapex-Dox agar medium was prepared and autoclaved. After cooling, the media was seeded with fungal strains and poured into sterile petri dishes. After solidification, 5 mm diameter holes were punched by a sterile cork-borer. After that, the extract was inoculated in petri dishes (only 0.1 mL), and the plates were incubated for 7 days at 30 °C. The activity was determined by measuring the diameter of the inhibition zone (in mm). The complex's percent activity index was calculated using the following equation:

$$\% \text{ Activity index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100$$

Cytotoxic activity

The human cancer cell lines (colon "HCT116", liver "HEPG2" and breast "MCF7") were obtained from the National Research Centre's tissue culture lab in Giza, Egypt. To assess the cytotoxicity of *G. barbadense* stem methanolic extract, The MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] use mitochondrial reductase to convert the water-soluble yellow dye to an insoluble purple formazan in 96-well plates [22]. The entire method was kept sterile by using a laminar air-flow cabinet and the culturing and sub-culturing approach developed by Alley *et al.* [23].

Statistical analysis

The statistical package for the social sciences (SPSS) version 23 was used to statistically analyze the data (copyrighted by IBM SPSS software, USA). The information was provided as a mean ± standard error of mean (SEM).

RESULTS AND DISCUSSION

Identification for essential oil components by headspace GC/MS

The petroleum ether fraction of *G. barbadense* stem is made up of 48 different compounds were recorded at (Figure 1 and Table 1), 13 of them are major compounds as indicated in (Figure 2); decane $C_{10}H_{22}$ (4.83%), 4-methyldecane $C_{11}H_{24}$ (2.67%), decahydronaphthalene $C_{10}H_{18}$ (2.83%), 2-methyldecane $C_{11}H_{24}$ (2.66%), 5,9,9-trimethylspiro [3.6] deca-5,7-dien-1-one $C_{13}H_{18}O$ (2.66%), undecane $C_{11}H_{24}$ (11.47%), 1-cyclohexyleicosane $C_{26}H_{52}$ (3.82%), tetralin $C_{10}H_{12}$ (3.10%), 2-methylundecane$C_{12}H_{26}$ (4.07%), dodecane$C_{12}H_{26}$ (7.63%), 2,6,10 trimethylpenta-decane $C_{18}H_{38}$ (2.77%), 6-methyltetralin $C_{11}H_{14}$ (3.45%)and 2-methyldodecane $C_{13}H_{28}$ (4.33%) which accounted for 56.29% of the total peak areas.

The quantitative composition and relative proportions of the oil components are heavily modified by genotype, ontogenic development, and environmental and growing factors or by plant species, chemotypes and climatic conditions. Given the differences in ecological and climatic conditions between Egypt and the rest of the world, this could explain the found compositional discrepancy between the plant under study and past research findings.

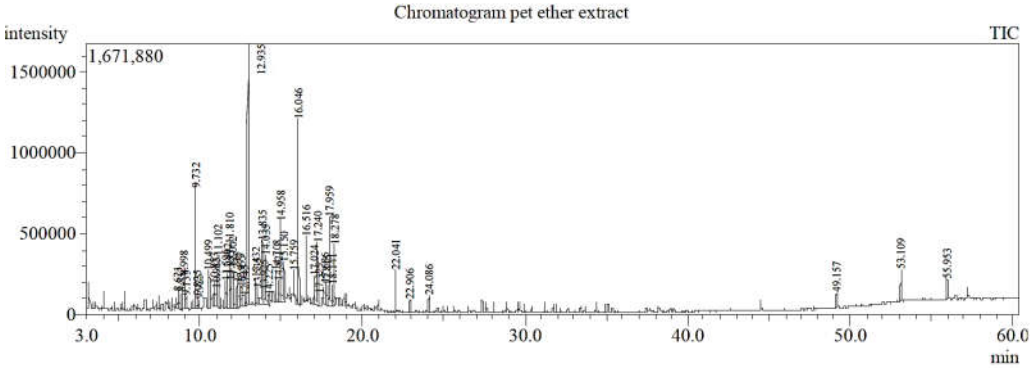

Figure 1. HS-GC/MS chromatogram of petroleum ether fraction *of G. barbadense* stem.

Table 1. HS-GC/MS Analysis of petroleum ether fraction of *G. barbadense* stem.

Peak	Compounds	R_t, min.	Area, %	Formula	Base peak m/z	Molecular weight
1	2-Methylnonane	8.621	0.81	$C_{10}H_{22}$	43.10	142
2	Di-n-Octyl ether	8.812	1.14	$C_{16}H_{34}O$	57.15	242
3	1,2,3-Trimethyl benzene	8.998	1.98	C_9H_{12}	105.15	120
4	1-Methyl-2-propylcyclohexane	9.135	0.59	$C_{10}H_{20}$	97.20	140
5	Decane	9.732	4.83	$C_{10}H_{22}$	43.1	142
6	1-Ethyl-4-methyl Benzene	9.825	0.57	C_9H_{12}	105.15	120
7	(1S,2R,5S)-4,6,6-Trimethylbicyclo [3.1.1] hept-3-en-2-yl decanoate	9.925	0.43	$C_{20}H_{34}O_2$	119.20	306
8	4-Methyldecane	10.499	2.67	$C_{11}H_{24}$	43.10	156
9	sec-Butylbenzene	10.853	1.14	$C_{10}H_{14}$	105.15	134
10	Sabinyl stearate	10.985	1.21	$C_{28}H_{50}O_2$	91.15	418
11	Decahydronaphthalene	11.102	2.83	$C_{10}H_{18}$	96.20	138
12	5-Methyldecane	11.596	1.08	$C_{11}H_{24}$	43.10	156
13	Butyric acid, 2-phenyl-, 2-ethylhexyl ester	11.692	1.89	$C_{18}H_{28}O_2$	119.20	276
14	2-Methyldecane	11.810	2.66	$C_{11}H_{24}$	43.10	156
15	*p*-Cymene	11.885	1.47	$C_{10}H_{14}$	119.20	134
16	5-n-Butylnonane	12.002	2.00	$C_{13}H_{28}$	43.10	184
17	Sulfurous acid, di (cyclohexyl methyl) ester	12.275	1.47	$C_{14}H_{26}O_3S$	97.20	274
18	5,9,9-Trimethylspiro [3.6] deca-5,7-dien-1-one	12.473	2.66	$C_{13}H_{18}O$	105.15	190
19	3,5,5,9-Tetramethyl-4a,5,6,7,8,9-hexahydro-2H-benzo[7] annulene	12.555	0.91	$C_{15}H_{24}$	119.20	204
20	2,4,4,6-Tetramethyl-6-phenyl-2-heptene	12.745	1.49	$C_{17}H_{26}$	119.20	230
21	Undecane	12.935	11.47	$C_{11}H_{24}$	43.10	156
22	1-Methylbicyclo (4.4.0) decane(trans)	13.432	1.23	$C_{11}H_{20}$	81.15	152
23	5-Methylundecane	13.535	1.17	$C_{12}H_{26}$	43.10	170
24	1-Cyclohexyleicosane	13.835	3.82	$C_{26}H_{52}$	83.15	364
25	m-Cymene	13.925	0.56	$C_{10}H_{14}$	119.20	134
26	Tetralin	14.035	3.10	$C_{10}H_{12}$	104.15	132
27	Hydratropic acid, undec-2-en-1-yl ester	14.225	1.07	$C_{20}H_{30}O_2$	105.15	302
28	Butyric acid, 2-phenyl-, dec-2-yl ester	14.708	1.96	$C_{20}H_{32}O_2$	119.20	304
29	4-Methylundecane	14.821	0.84	$C_{12}H_{26}$	43.10	170
30	2-Methylundecane	14.958	4.07	$C_{12}H_{26}$	43.10	170
31	Oxalic acid, bis(6-ethyloct-3-yl) ester	15.150	2.31	$C_{22}H_{42}O_4$	85.20	370
32	Methyl tetralin	15.759	1.18	$C_{11}H_{14}$	104.15	146

33	Dodecane	16.046	7.63	$C_{12}H_{26}$	43.10	170
34	2,6,10-Trimethylpentadecane	16.516	2.77	$C_{18}H_{38}$	57.15	254
35	1-Cyclohexylhexane	17.024	1.28	$C_{12}H_{24}$	83.15	168
36	6-Methyltetralin	17.240	3.45	$C_{11}H_{14}$	118.20	146
37	n-Hexyl benzene	17.325	0.60	$C_{12}H_{18}$	91.10	162
38	Sulfurous acid, hexyl pentadecyl ester	17.686	1.62	$C_{21}H_{44}O_3S$	43.10	376
39	4-Methyldodecane	17.815	1.21	$C_{13}H_{28}$	43.10	184
40	2-Methyldodecane	17.959	4.33	$C_{13}H_{28}$	43.10	184
41	10-Methylnonadecane	18.141	0.94	$C_{20}H_{42}$	57.15	282
42	4,6-Dimethyldodecane	18.278	2.49	$C_{14}H_{30}$	71.15	198
43	4,8,8-Trimethyl-2-methylene-4-vinylbicyclo[5.2.0] nonane	22.041	1.95	$C_{15}H_{24}$	93.15	204
44	1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	22.906	0.59	$C_{15}H_{24}$	93.15	204
45	2,4-Di-tert-butylphenol	24.086	0.71	$C_{14}H_{22}O$	191.20	206
46	2,6,10,15,19,23-hexamethyl-, (all-E)-(+/-)-1,6,10,14,18,22-Tetracosahexaen-3-ol	49.157	0.62	$C_{30}H_{50}O$	81.15	426
47	dl-α-Tocopherol	53.109	1.70	$C_{29}H_{50}O_2$	165.15	430
48	β-Card-20(22)-enolide,3.β,5,14,19-tetrahydroxy	55.953	1.53	$C_{23}H_{34}O_6$	107.15	406

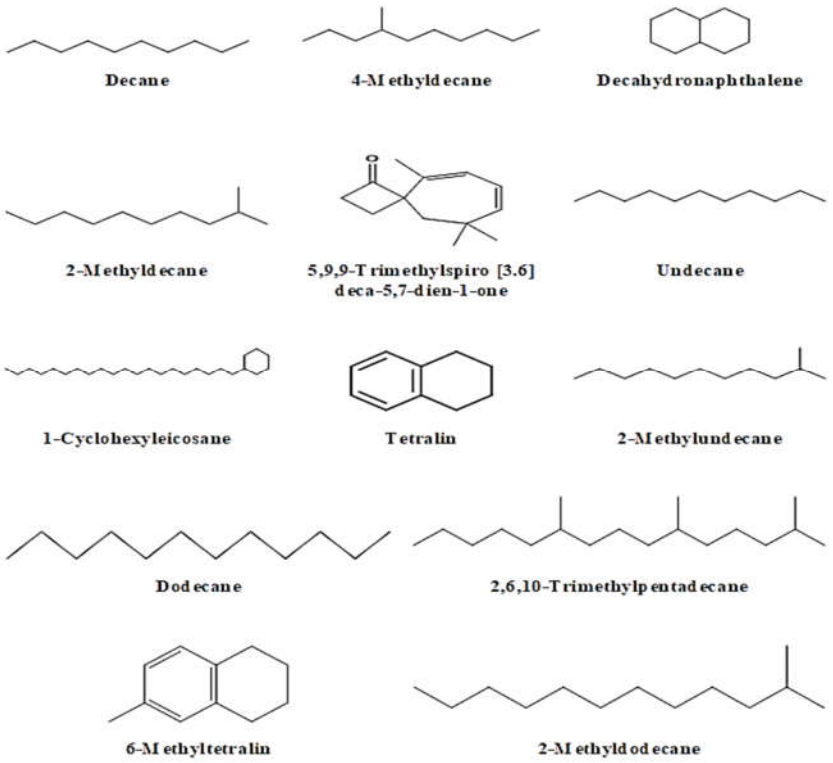

Figure 2. The major compounds identified by HS-GC/MS chromatogram of petroleum ether fraction of *G. barbadense* stem.

Identification of phenolic and flavonoid compounds of methanolic extract by using LC-ESI-MS analysis

The analysis of methanolic extract from *G. barbadense* stem indicates the presence of 16 major components as follows: cinnamaldehyde, cinnamyl alcohol, kaempferol 4'-methyl ether, (+/-)-catechin, kaempferol-3-sulfate, isoquercetin, quercetin-3-*O*-(6-acetyl-*β*-glucoside), *p*-cresol-sulfate, epigallocatechin gallate, hydroxybenzoic acid, haploperoside *C*, kaempferol-3,7,4'-trimethyl ether, flavone base + 3*O*, 2MeO, protocatechuic acid-3-*O*-glucoside and flavanone base + 4*O*, *O*-hex, C-pen were recorded at (Table 2).

All identified phenolic compounds from methanolic extract of *G. barbadense* stem are therapeutically active compounds with anticancer potency and antimicrobial capacities which were reported for the first time.

Table 2. Major bioactive phenolic compounds identified by LC-ESI-Mass *of G. barbadense* stem MeOH extract.

Peak no	R_t (min)	Area (%)	Exact mass (g/mol)	Chemical formula	Compounds
1	1.40	7.46	132.05751	C_9H_8O	Cinnamaldehyde
2	1.56	12.53	134.17799	$C_9H_{10}O$	Cinnamyl alcohol
3	2.26	0.92	300.26599	$C_{16}H_{12}O_6$	Kaempferol 4'-methyl ether
4	2.70	0.74	290.07904	$C_{15}H_{14}O_6$	(+/-)-Catechin
5	8.27	13.43	366.17053	$C_{15}H_{10}O_9S$	Kaempferol -3-Sulfate
6	9.06	7.45	464.37900	$C_{21}H_{20}O_{12}$	Isoquercetin
7	9.50	1.43	506.10605	$C_{23}H_{22}O_{13}$	Quercetin 3-*O*-(6-acetyl-*β*-glucoside)
8	10.50	1.46	188.01433	$C_7H_8O_4S$	*p*-Cresol sulfate
9	11.77	5.02	458.08490	$C_{22}H_{18}O_{11}$	Epigallocatechin gallate
10	12.61	3.54	138.03169	$C_7H_6O_3$	Hydroxybenzoic acid
11	13.11	1.41	500.15298	$C_{22}H_{28}O_{13}$	Haploperoside *C*
12	13.89	5.77	328.09470	$C_{18}H_{16}O_6$	Kaempferol-3,7,4'-trimethyl ether
13	15.05	6.94	330.07394	$C_{17}H_{14}O_7$	Flavone base + 3O, 2MeO
14	15.79	1.14	492.38901	$C_{22}H_{20}O_{13}$	Isorhamnetin-3-*O*-glucuronide
15	19.81	1.51	316.26501	$C_{13}H_{16}O_9$	Protocatechuic acid-3-*O*-glucoside
16	29.06	5.13	582.51099	$C_{26}H_{30}O_{15}$	Flavanone base + 4O, O-Hex, C-Pen

Antimicrobial activity

Microbial infections have become more common in recent decades, particularly bacterial, which cause a high rate of death in immunocompromised patients. Opportunistic bacterial infections pose a significant risk to these individuals and have been documented to occur at an alarming rate [25]. The limited number of antimicrobial medicines available, the accompanying adverse effects of amphotericin B (i.e., nephrotoxicity), the high cost of amphotericin B lipid formulations and the potential failure of some persons to react to azole treatment may all complicate treatment of these microbial. As a result, there is an urgent need to discover new antimicrobial medicines or chemicals [26].

Plant-based antibacterial compounds have tremendous therapeutic potential since they can accomplish the purpose without or with minimal adverse effects that are frequently linked with manufactured medicines. Plants are a rich source of potentially beneficial structures for developing novel chemotherapeutic drugs. Diffusion agar was used to test the antibacterial and

antifungal properties of petroleum ether and methanolic extracts of *G. barbadense* stem. The mean zone of inhibition in mm produced on a variety of harmful bacteria was measured and recorded in (Table 3). The results were compared to the activity of commercially available standard antibiotic (ciprofloxacin).

Table 3. Antimicrobial activities of different extracts from *G. barbadense* stem.

Extracts / Microorganisms	Petroleum ether extract		Methanolic extract		Standard antibiotics (Ciprofloxacin)	
	Diameter of inhibition zone (mm)	Activity index (%)	Diameter of inhibition zone (mm)	Activity index (%)	Diameter of inhibition zone (mm)	Activity index (%)
Staphylococcus aureus ATCC6538	10	50%	35	175%	20	100
Escherichia coli ATCC10536	28	93.33%	45	150%	30	100
Pseudomonas aeruginosa ATCC 9027	10	35.71%	NA	--	28	100
Salmonella enterica ATCC 14028	NA	--	15	88.24%	17	100
Aspergillus niger	NA	--	NA	--	NA	--
Aspergillus fumigatus	NA	--	NA	--	NA	--

NA: No activity.

Methanolic extract showed high antibacterial activity against *S.aureus* (35 mm), while the petroleum ether extract showed lower activity than methanol extract (10 mm). Also, methanolic extract revealed significant antibacterial activity against *E. coli* (45 mm) compared to petroleum ether extract (28 mm). Petroleum ether extract showed antipseudomonal activity (10 mm), while methanol extract revealed no activity. On the other hand, the methanolic extract exhibited antibacterial activity against *S. enterica* (15 mm), while petroleum ether had no activity. However, neither petroleum ether extract nor methanolic extract of *Gossypium barbadense* stem had antifungal activity against *A. niger* or *A. fumigatus*.

Moreover, the petroleum ether extract showed moderate antibacterial activity against *S. aureus* (50%), strong antimicrobial activity against *E. coli* (93.33%), and weak antimicrobial activity against *P. aeruginosa* (35.71%) when compared to ciprofloxacin. This finding may provide validity to the antibacterial activities of this species' extracts. The major components or synergy between major and minor molecules may be responsible for an essential's antibacterial activity.

On the other hand, the methanolic extract displayed more antimicrobial activity than Ciprofloxacin against *S. aureus* (175%) and *E. coli* (150%). Furthermore, the high activity of methanolic extract could be attributed to two factors. To begin, the bioactive ingredients detected by LC-ESI-MS analysis in the plant extract, such as phenolics and flavonoids, may be amplified in the presence of methanolic extract. Second, the higher extraction capacity of methanolic extract may be to blame, resulting in more active chemicals in the polar extracts. Some of the compounds found in this extract may be responsible for the observed antibacterial activity, supporting their historic use as medicinal herbs for the treatment of bacterial gastroenteritis [27]. Chah *et al.* [28] revealed that a methanolic extract of dried fresh *G. barbadense* leaves had dose-dependent efficacy against four wound isolates (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis* and *Shigella sonnei*) using the well diffusion method. Inaddition, *Gossypium hirsutum* leaf extracts shown antibacterial efficacy against clinically relevant pathogens such as *E. coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Shigella dysenteriae*. The antibacterial activity of ethanolic extract was greater than that of water extract, *Shigella dysenteriae* was the

most sensitive microbe (13 mm), and *Pseudomonas aeruginosa* was the least sensitive (6 mm). The minimum inhibitory concentration against all the microorganisms tested was 0.25 (% w/v) [29].

In vitro cytotoxic activity

The cytotoxic impact of *G. barbadense* stem methanolic extract on three human cancer cell lines (HCT116, HEPG2 and MCF-7) was assessed using doxorubicin as a positive control and untreated cells as a negative control. By comparison the resulted data in (Table 4) with doxorubicin, the MeOH extract showed potent effect against HEPG2 with IC_{50} values (18.8 ± 7.4 µg/mL), whilst the cytotoxicity effect on HCT116 and MCF-7 were considered moderate activity with IC_{50} values (50 ± 6.2 and 39 ± 5.6 µg/mL), respectively.

Table 4. In *vitro* cytotoxic activity of methanolic extract of *G. barbadense* stem towards human colon (HCT116), liver cancer (HEPG2) and Breast (MCF7) cell lines.

Extract	Colon (HCT116) IC_{50} [µg/mL]	Liver (HEPG2) IC_{50} [µg/mL]	Breast (MCF7) IC_{50} [µg/mL]
Methanolic extract	50 ± 6.2	18.8 ± 7.4	39 ± 5.6
Doxorubicin	4.8 ± 0.6	2.7 ± 0.06	5.03 ± 0.7
DMSO	N.A.	N.A.	N.A.

The anticancer activity of *G. barbadense* stem methanolic extract can be linked to its high flavonoid and phenolic content. The flavonoids inhibit cancer cell proliferation and tumor growth. Compounds, catechin, kaempferol-3-sulfate, and isoquercetin, the hydroxylation pattern of the flavanone and flavanol B ring appears to have a substantial effect on their actions, particularly protein kinase inhibition and antiproliferation [30]. The powerful action of methanolic extract is attributed to its high constituents of isolated phenolic and flavonoid compounds with various hydroxy groups in the flavonoid structure, which, when combined with a strongly conjugated electron system, allows them to act as free radical scavengers *via* hydrogen atom or electron donation activities. Furthermore, by chelating redox-active transition metal ions, they can prevent the generation of ROS (reactive oxygen species) such as hydroxyl radicals [31]. By minimizing events like DNA oxidative damage, this has the potential to improve cancer diagnosis.

The present anticancer agents are amazingly cytotoxic and exhibit serious adverse effects on various tissues of human at clinical level. As a result, these restrictions on the use of synthetic anticancer compounds would necessitate the screening and development of new medicinal plant extracts as alternatives that are less toxic to normal cells, have a higher therapeutic index, a different mechanism of action, and shorter treatment cycles. According to the findings of the current study, "*G. barbadense* crude extract clearly demonstrated a cytotoxic impact on cancer cell lines of various types, with greater potency than normal cell lines" .This substance has been utilized as a male oral contraceptive [32] and shows anticancer action, specifically against melanoma and bladder carcinoma [33].

According to certain reports, gossypol is more cytotoxic in specific tumor cell lines [34]. The precise biochemical and/or molecular pathways involved in the progression of such antiproliferative/cytotoxic biological action are not well understood. Given the essential processes in cancer cell death, significant efforts are being undertaken to translate this knowledge into reasonable design and development of novel treatment techniques to improve the efficacy of chemotherapeutic drugs [35]. The current investigation found that the methanolic extract had a cytotoxic impact on HEPG2 and MCF7 cancer cell lines, with the exception of HCT 116, which was resistant (IC_{50} value 50 ± 6.2 µg/mL).

CONCLUSION

The present study has found that petroleum ether extract of *G. barbadense* stem revealed 48 compounds; thirteen of them are significant and account for more than 55% of the total components using HS-GC/MS analysis. In addition, the methanolic extract of *G. barbadense* stem indicated the presence of 16 major compounds from phenolics and flavonoids were identified by using LC-ESI-MS, both analysis for the first time. Based on these findings, it is concluded that methanolic extract is a more promising candidate than petroleum ether extract against pathogenic bacterial strains. Furthermore, it was possible to establish that the methanolic extract of *G. barbadense* stem exhibited a high antiproliferation/cytotoxicity index against liver cancer cell lines.

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