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Full Length Research Paper

Chemical constituents and biological activities of different solvent extracts of *Prosopis farcta* growing in Egypt

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Different solvent extracts from the aerial part of *Prosopis farcta* growing in Egypt have been biologically evaluated by studying their antimicrobial, anticancer and antioxidant activities. Furthermore, the chemical analysis using GC/MS has been performed for the promising extracts *n*-hexane and methylene chloride, and this analysis led to the identification of twenty six and thirty two compounds respectively from *n*-hexane and methylene chloride. The major compound identified in the *n*-hexane is (Z) 9,17-octadecadienal (10.60%) while for methylene chloride is tricosanoic acid (9.24%). In addition, chromatographic isolation of the ethyl acetate and *n*-butanol extracts resulted in the isolation of four compounds which were identified as; dihydrokaempferol-3-*O*-α-L-rhamnoside (1), apigenin (2), 4′-methoxyquercetin (tamarixetin) (3) and acacetin-7-*O*-α-L-rhamnoside (4). *n*-hexane and methylene chloride showed moderate antimicrobial activities against three microbes for each, that is, *Shigella* spp., *Escherichia coli* and *Proteus vulgaris* for *n*-hexane and *Erwinia spp.*, *Escherichia coli* and *Staphylococcus epidermis* for methylene chloride. On the other hand, the ethyl acetate showed higher antimicrobial activities against *Shigella* spp., *Escherichia coli*, and *Candida albicans*. Likewise *n*-butanol extract showed higher activity against *Shigella* spp., *Erwinia spp., E. coli*, *P. vulgaris*, *S. epidermis* and *Candida albicans*. Moreover, the anticancer activities were evaluated against four human tumor cell lines namely; HepG-2, HeLa, PC3 and MCF-7. The *n*-butanol extract showed the highest activity against MCF-7 cell line with IC_{50} of 5.6 µg/ml compared to 5-fluorouracil with IC_{50} of 5.4 µg/ml, while the ethyl acetate showed the highest activity against Hela cell line with IC_{50} of 6.9 µg/ml compared to 5-fluorouracil with IC_{50} of 4.8 µg/ml. Also, the inhibition percentages (I%) of ABTS radical were 83.1, 82.0, 87.2 and 87.0% respectively for the *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol extracts, respectively, compared to ascorbic acid with 89.2%. In, conclusion the different extracts of *P. farcta* aerial part showed promising antimicrobial, anticancer and antioxidant activities, in which may be return to their identified bioactive secondary metabolites.

Key words: *Prosopis farcta*, antimicrobial, anticancer, antioxidant, chemical constituents.

INTRODUCTION

Due to, the rapid development of drug-resistant micro-organisms, appearance of new patients of life-threatening infections and the constant return of diseases, the search for the discovery of new drugs got strong attention from researchers (Demain, 2000; Strobel et al., 2004). So, it is necessary to concentrate on the antimicrobial activity of plant derived substances that are being still used as global traditional medicine (Savoia, 2012). Plant secondary metabolites are responsible for the antimicrobial activity. Vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), multi-drug resistant (MDR)- *Mycobacterium tuberculosis* and MDR Gram-negative bacteria are documented as the most complicated infections to overcome and heal (Dahiya and Purkayastha, 2012). Increased illness and death, especially in the third world countries, have been due to the development of broad-spectrum β-lactamases (ESBLs) and carbapenemases that target Gram-negative bacteria (WHO, 2002). To overcome the resistance problem, there is a command need to do a renewed effort to screen various medicinal plants for their potential antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. Alkaloids, flavonoids, tannins, phenolic compounds, steroids, resins, fatty acids and gums are the most important of these bioactive compounds of plants which are capable of producing specific physiological effect on body. Medicinal plants are used to treat about 80% of the world's population especially in India. The plant materials used in traditional medicine are considered more effective and relatively cheaper than manufactured medicine (Iwu et al., 1999; Mann et al., 2008). On the other hand, anticancers were isolated from different plant species. The first anticancer agents introduced in clinical use were vinca alkaloids, that is, vinblastine (VLB) and vincristine (VCR) isolated from *Catharanthus roseus* (Parakash et al., 2013).

The genus *Prosopis* L., is widely spread in different dry and semi-dry regions worldwide like; Africa, Australia, America and Asia (Perez-Garcia et al., 2012; Peña-Avelino et al., 2014; Abdelmoteleb et al., 2017). Approximately, this genus comprises from 44 to 50 species (William and Jafri, 2015). *Prosopis farcta* (Banks et Sol.) Eig. (Family Fabaceae) is a little prickly spiny shrub; it is native of United States, Kuwait, Turkey, Iraq, Iran, Northern Africa, and South Western Asia (Sharifi-Rad et al., 2014). Different phytochemicals were reported to be isolated and identified from the plant *viz*., volatile constituents (Harzallah-Skhiri et al., 2006), proteins and unsaturated fatty acids (Lajnef et al., 2015), and flavonoids (Direkvand-Moghadam et al., 2014). A literature survey revealed that *P. farcta* has been used in the treatment of neurological disorders (Mollashahi et al., 2013) and cholesterol level (Omidi et al., 2013). Moreover, different parts of the plant (seeds, pods and leaves) were reported to show a wide range of biological activities including; antioxidant (Poudineh et al., 2015), antibacterial (Sharifi-Rad et al., 2014), hepatoprotective (Alharbi et al., 2017), anti-diabetic (Jafari et al., 2013), to reduce cardiac pain (Asadollahi et al., 2010), and antiparasitic (Gulalp and Karcioglu, 2008). To the best of our knowledge, there is little information in the literature about the chemical and biological investigations were carried out on the aerial part of *P. farcta* growing in Egypt. Therefore, the present study is aimed at the isolation and identification of the chemical constituents from different solvent extracts of *P. farcta* aerial part, and evaluation of their antimicrobial, anticancer and antioxidant activities.

MATERIALS AND METHODS

Plant material

The aerial parts of *P. farcta* (Banks et Sol.) Eig. (Fabaceae) were collected from Alkharga Oasis, Alwady Algaded, Egypt on March, 2015. The plant was identified by Prof. Dr. Ibrahim A. Mashaly, Professor of Plant Ecology and Flora, Botany Department, Faculty of Science, Mansoura University, Egypt.

Extraction and fractionation

The aerial parts were dried in an oven at 45°C for 24 h and been grinded to give 1.5 kg of dried powder. Then, powdered materials were soaked in MeOH for 72 h, at room temperature. After filtration, the solvent was evaporated using rotatory evaporator, resulting crude extract that was undergo further solvent extraction using different organic solvents that is, *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol.

Antimicrobial activity

The antimicrobial activity was evaluated by filter paper disc method (Murray et al., 1998; Sardari et al., 1998). Briefly, filter paper discs, 5 mm diameter, were saturated with 200 µg of tested extract. Stock cultures of the test organisms were obtained from the Microbiological Laboratory, Faculty of Medicine, Mansoura University. Bacteria test microbes used were *S. aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia, Escherichia coli*, *Bacillus subtilis*, *Erwenia carotovora*, *Shigella* sp., *Erwinia* sp., *Enterobacter aerogenes*, *Pseudomonas aeruginosa, Proteus vulgaris* and *Staphylococcus epidermis.* Whereas the fungus used was *Candida albicans.* The bacterial test microbes (10^6 cells/ml)

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were swapped on plates containing nutrient agar medium (DSMZ1) whereas, the fungus test microbe (10^8 cell/ml) was swapped on plates containing Czapek-Dox medium (DSMZ130). The filter paper discs-containing the tested extracts were put on the surfaces of the inoculated plates. The plates were then incubated at 37 and 30°C, for bacteria and fungus test microbes respectively. The appearance of clear zones (mm diameter) was detected after 24 h of incubation. The activity index (%) is also measured as a correlation of the clear zone of tested extract compared to standard antibiotics (Ampicillin, Streptomycin, Kanamycin, Tobarmycin and Clotrimazole). The activity index was measured according to the following equation:

$$\%\,Activity\ Index = \frac{Zone\ of\ inhibition\ by\ test\ compound\ (diametre)}{Zone\ of\ inhibition\ by\ standard\ (diametre)} x100$$

Anticancer activity (MTT assay)

The anticancer activity was evaluated according to the reported procedure (Mauceri et al., 1998), using four human tumor cell lines namely; hepatocellular carcinoma (HePG-2), mammary gland breast cancer (MCF-7), human prostate cancer (PC3), and Epitheliod carcinoma (Hela). The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. 5-fluorouracil was used as a standard anticancer drug for comparison. Briefly, the different cell lines mentioned above were used to determine the inhibitory effects of extracts/compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100 µg/ml streptomycin at 37°C in a 5% CO_2 incubator. The cells were seeds in a 96-well plate at a density of 1.0x104 cells/well at 37°C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5 mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100.

Antioxidant activity (ABTS assay)

The antioxidant activity was evaluated via 2,2-azino-di-[3-ethylbenzo-thiazolin-sulphonate] (ABTS) method. Briefly, for each of the investigated sample 2 mL of ABTS solution (60 mM) was added to 3 M MnO_2 solution (25 mg/ml) all prepared in phosphate buffer (pH 7, 0.1 M). The mixture was shaken, centrifuged, filtered, and the absorbance ($A_{control}$) of the resulting green-blue solution (ABTS radical solution) was adjusted at ca. 0.5 at 734 nm. Then, 50 ml of (2 mM) solution of the test compound in spectroscopic grade MeOH/ phosphate buffer (1:1) was added. The absorbance (A_{test}) was measured and the reduction in color intensity was expressed as % inhibition. The % inhibition for each compound is calculated from the following equation:

% Inhibition = $\mathbf{A_{control}\text{-}A_{test}/A_{control}\ x\ 100}$

Ascorbic acid (vitamin C) was used as standard antioxidant (positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of sample. Negative control sample was run with MeOH/phosphate buffer (1:1) instead of tested sample (El-Gazzar et al., 2009).

GC/MS analysis

GC/MS analysis was performed at the Central Laboratory of the Ministry of Agriculture, Al-Bhooth Str., Cairo, on Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric column PAS-5ms (30 m x 0.32 mm x0.25 um film thickness). The plant extracts were injected under the following conditions. Helium was used as carrier gas at approximately 1.0 ml /min, pulsed splitless mode. The solvent delay was 3 min, and the injection size was 1.0 µl. The mass spectrophotometric detector was operated in electron impact ionization mode an ionizing energy of 70 eV scanning from *m/z* 50 to 500. The ion source temperature was 230°C and the quadrupole temperature was 150°C. The electron multiplier voltage (EM voltage) was maintained at 1250 V above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 60°C then elevated to 280°C at rate of 8°C/min and 10 min hold at 280°C the detector and injector temperature were set at 280 and 250°C, respectively. Wiley and Nist 05 mass spectral data base was used in the identification of the separated peaks.

Chromatographic isolation of EtOAc and *n*-BuOH extracts

The ethyl acetate extract (3.12 g) was subjected to polyamide column chromatography, initially eluted with distilled water (H_2O), followed by gradient mix elution with methanol, acetic acid and ammonia to afford one major fraction **(I)**, further purification of **(I)** over Sephadex LH-20 column chromatography eluted with (CH_2Cl_2: MeOH; 1:9; v/v) followed by extra purification over preparative thin layer chromatography (PTLC) (EtOAc: MeOH; 3.1:1.9; v/v) led to the isolation of compound **1**. Moreover, the *n*-butanol extract (4.09 g) was subjected to polyamide column chromatography, initially eluted with distilled water (H_2O), followed by gradient mix elution with methanol, acetic acid and ammonia to afford three major fractions **(II, III** and **IV)**.The first fraction **(II)** was eluted in MeOH and resubmitted to further purification over Sephadex LH-20 column chromatography eluted with (CH_2Cl_2: MeOH; 1:9; v/v) followed by extra purification over preparative thin layer chromatography (PTLC) (EtOAc: MeOH; 2.8:2.2; v/v) to afford compound **2**. The second fraction **(III)** was eluted in MeOH+AcOH and undergoing further purification over Sephadex LH-20 column chromatography eluted with (CH_2Cl_2: MeOH; 1:9; v/v) followed by extra purification over preparative thin layer chromatography (PTLC) (EtOAc: MeOH; 3:2; v/v) to afford compound **3**. Finally, the third fraction **(IV)** was eluted in AcOH and resubmitted to further purification over Sephadex LH-20 column chromatography eluted with (CH_2Cl_2: MeOH; 1:9; v/v) followed by extra purification over preparative thin layer chromatography (PTLC) (EtOAc: MeOH: H_2O; 9:0.8:0.2; v/v/v) to afford compound **4**. The chemical structures of the four isolated compounds are shown in Figure 1.

RESULTS AND DISCUSSION

Identification and characterization of the isolated compounds (1-4)

Dihydrokaempferol-3-*O*-α-L-rhamnoside (1): Was obtained as white powder, m.p. 282°C, Rf 0.56 in (15% AcOH; PC) and 0.34 in (EtOAc: MeOH; 3.1:1.9; TLC). It showed a dark purple spot upon paper chromatography under UV light. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ in ppm:

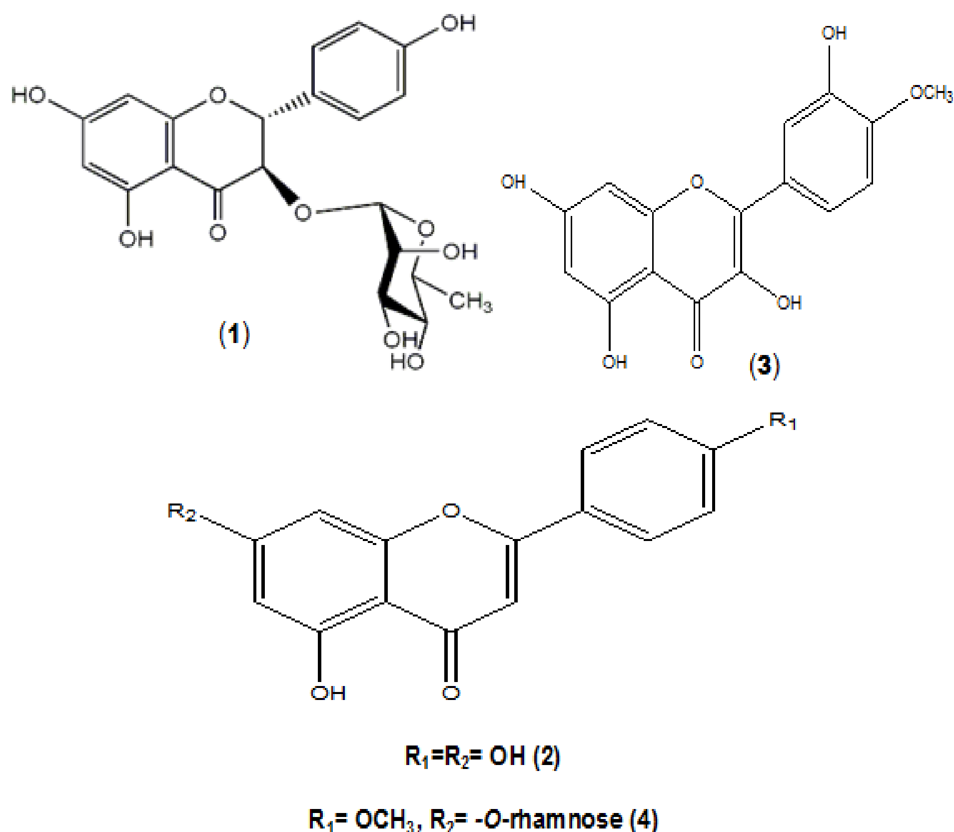

Figure 1. Chemical structures of pure compounds were isolated from the EtOAc and *n*-BuOH extracts of *P. farcta*.

7.73 (2H, *d*, *J*= 8.8 Hz, H-2',6'), 6.94 (2H, *d*, *J*= 8.8 Hz, H-3',5'), 6.49 (1H, *d*, *J*= 2.0 Hz, H-6), 6.18 (1H, *d*, *J*= 2.0 Hz, H-8), 4.73 (1H, *d*, *J*= 10.8 Hz, H-2), 4.60 (1H, *d*, *J*= 10.8 Hz, H-3), 4.14 (1H, *d*, *J*= 2.0 Hz, Rha-1''), 0.90 (3H, *d*, *J*= 7.2 Hz, CH_3) and 3.31-3.79 ppm (m, rest of sugar protons). Complete acid hydrolysis of compound 1 revealed the presence of dihydrokaempferol as aglycone (Co-PC) and rhamnose as sugar moiety (Co-TLC). Moreover, all protons signal (chemical shifts and coupling constants) were matched with the reported in the literature (Fujiwara et al., 2011; Xiao et al., 2011).

Apigenin (2): Was obtained as faint yellow powder, m.p. 358°C, Rf 0.05 in (15% AcOH; PC) and 0.29 in (EtOAc: MeOH; 2.8:2.2; TLC).It showed a dark purple spot upon paper chromatography under UV light without any change after spraying with $AlCl_3$. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ in ppm revealed the presence of two doublet of doublets at 7.91 (2H, *d*, *J*= 8.4 Hz, H-2',6') and 6.94 ppm (2H, *d*, *J*= 8.4 Hz, H-3',5'), a singlet signal at 6.75 ppm (1H, s, H-3), two meta coupled aromatic doublets characteristic for ring-A at 5.97 (1H, *d*, *J*= 2.0 Hz, H-6) and 6.49 ppm (1H, *d*, *J*= 2.0 Hz, H-8), these spectral data were characteristic for a 5,7,4'-trisubstituted flavone skeleton which was confirmed via Co-paper with apigenin authentic sample (identical R_f and spot colour). Moreover, spectral data of compound 2 were in agreement with the reported data (Fujiwara et al., 2011).

4′-methoxyquercetin (tamarixetin) (3): Was obtained as yellow powder, m.p. 216°C, Rf 0.09 in (15% AcOH; PC) and 0.41 in (EtOAc: MeOH; 3:2; TLC). It showed yellow spot upon paper chromatography under UV light. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ in ppm revealed the presence of three characteristic signals for three aromatic protons at 7.80 (1H, *d*, *J*= 8.6 Hz, H-2'), 7.50 (1H, *dd*, *J*= 8.6, 2.4 Hz, H-6'), and 6.90 ppm (1H, *d*, *J*= 8.6 Hz, H-5') for ring-B. Also, two meta coupled protons at 6.65 (1H, *d*, *J*= 2.4 Hz, H-8), and 6.32 ppm (1H, *d*, *J*= 2.4 Hz, H-6) for ring-A. The appearance of one signal for methoxyl group at 3.80 (3H, -OCH_3), spectral data of compound 3 were in agreement with the reported data (Awaad et al., 2012).

Acacetin-7-*O*-α-L-rhamnoside (4): Was obtained as yellow powder, m.p. 260°C, Rf 0.48 in (15% AcOH; PC) and 0.52 in (EtOAc: MeOH: H_2O; 9:0.8:0.2; TLC). It showed dark purple spot upon paper chromatography under UV light. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ in ppm revealed the presence of two doublet signals at 7.93 (2H, *d*, *J*= 8.6 Hz, H2',6') and 6.94 ppm (2H, *d*, *J*= 8.6 Hz,

Table 1. The compounds identified in the *n*-hexane extract of *P. farcta* by GC/MS analyses.

R.T.	No.	Compound Name	Area%	M.F	Molecular ion peak, M^+		Base peak
					m/z	%	
12.31	1	Thiophene, tetrahydro-, 1,1-dioxide	0.76	$C_4H_8O_2S$	120	60.24	41
17.10	2	Tetradecane	0.30	$C_{14}H_{30}$	198	3.21	57
19.57	3	Pentadecane	0.75	$C_{15}H_{32}$	212	3.03	57
21.62	4	Dodecanoic acid	0.36	$C_{12}H_{24}O_2$	200	7.24	119
21.74	5	1-Tridecene	0.33	$C_{13}H_{26}$	182	4.65	55
23.00	6	4-methyl-14-pentadecenoic acid	3.46	$C_{16}H_{30}O_2$	254	2.04	91
26.11	7	1-Icosene	0.58	$C_{20}H_{40}$	280	1.65	55
26.26	8	Octadecane	0.78	$C_{18}H_{38}$	254	3.65	57
28.26	9	Nonadecane	0.79	$C_{19}H_{40}$	268	2.84	57
28.83	10	Pentadecanoic acid	1.67	$C_{15}H_{30}O_2$	242	2.97	74
30.05	11	Nonadecanoic acid	9.10	$C_{19}H_{38}O_2$	298	1.95	73
32.02	12	Henicosane	1.06	$C_{21}H_{44}$	296	3.19	57
32.36	13	*trans*-Phytol	1.39	$C_{20}H_{40}O$	296	1.97	71
32.57	14	Methyl icosanoate	0.21	$C_{21}H_{41}O_2$	326	2.47	74
33.25	15	(Z)9,17-Octadecadienal	10.60	$C_{18}H_{32}O$	264	15.67	55
33.54	16	stearic acid	0.47	$C_{18}H_{36}O_2$	284	16.98	55
33.80	17	Docosane	0.25	$C_{22}H_{46}$	310	4.16	57
37.10	18	Tetracosane	0.73	$C_{24}H_{50}$	338	2.12	57
38.66	19	Pentacosane	0.29	$C_{25}H_{52}$	352	1.94	57
40.16	20	Hexacosane	0.26	$C_{26}H_{54}$	366	1.08	57
41.61	21	Heptacosane	0.15	$C_{27}H_{56}$	380	2.87	57
43.47	22	Supraene	0.25	$C_{30}H_{50}$	410	1.69	69
47.58	23	dl-α-Tocopherol	6.23	$C_{29}H_{50}O_2$	430	100	430
49.81	24	γ-Sitosterol	0.51	$C_{29}H_{50}O$	414	100	414
50.80	25	Taraxasterol	0.81	$C_{30}H_{50}O$	426	50.62	207
50.93	26	Urs-20-en-16β-Ol.	0.53	$C_{30}H_{50}O$	426	12.97	189
Total %			**42.62%**				

H3',5'), a singlet at 6.50 ppm (1H, s, H-3) and two meta coupled protons at 6.41 (1H, *d*, *J*= 2.0 Hz, H-8) and 6.37 ppm (1H, *d*, *J*= 2.0 Hz, H-6), anomeric proton of rhamnosyl moiety at 5.35 ppm (1H, brd, Rha-1''), methoxy group at 4.04 ppm (3H, $-OCH_3$), 3.83-3.25 (m, rest of sugar protons) and methyl protons of the rhamnosyl moiety were appeared at 1.23 ppm (3H, *d*, *J*= 6.4, Rha-CH_3). Moreover, complete acid hydrolysis of compound 4 revealed the presence of acacetin as aglycone (Co-PC) and rhamnose as sugar moiety (Co-TLC). All protons resonances of compound 4 were in agreement with those reported by Rashed et al. (2015).

GC/MS analyses of the *n*-hexane and methylene chloride extracts

The chemical constituents of the *n*-hexane extract are shown in (Table 1). These constituents were identified using GC/MS analysis which revealed the presence of twenty six compounds representing 42.62% of the total extract composition. (Z)9,17-octadecadienal (10.60%), nonadecanoic acid (9.10%), dl-α-tocopherol (6.23%) and 4-methyl-14-pentadecenoic acid (3.46%) were the major components (Figure 2). On the other hand, the chemical constituents of the methylene chloride extract are shown in (Table 2), which revealed the presence of thirty two compounds representing 85.34% of the total extract composition. Tricosanoic acid (9.24%), (Z,Z)-9,12-octadecadienoic acid (9.10%), 3-hydroxy-β-damascone (6.53%), 1,2-dihydrophenanthrene-4-carboxylic acid (5.54%), and trans-piperitone oxide (5.11) were the major components (Figure 3). Reviewing the literature revealed that there is no any reported data about the GC/MS analyses of *P. farcta* extracts, but the available data were reported on the volatile constituents of roots, leaves, pods, flowers and branches parts of *P. farcta* growing in Tunisia, the major compounds were identified in the root part are octadecanal (10.5%), hexadecanal (9.0%), heptadeca-1, 11,13-triene (8.3%) and octadeca-1-ene (7.0%); while for the branch part are 6,10,14-trimethylpentadecan-2-one (8.6%), D-limonene (5.4%)

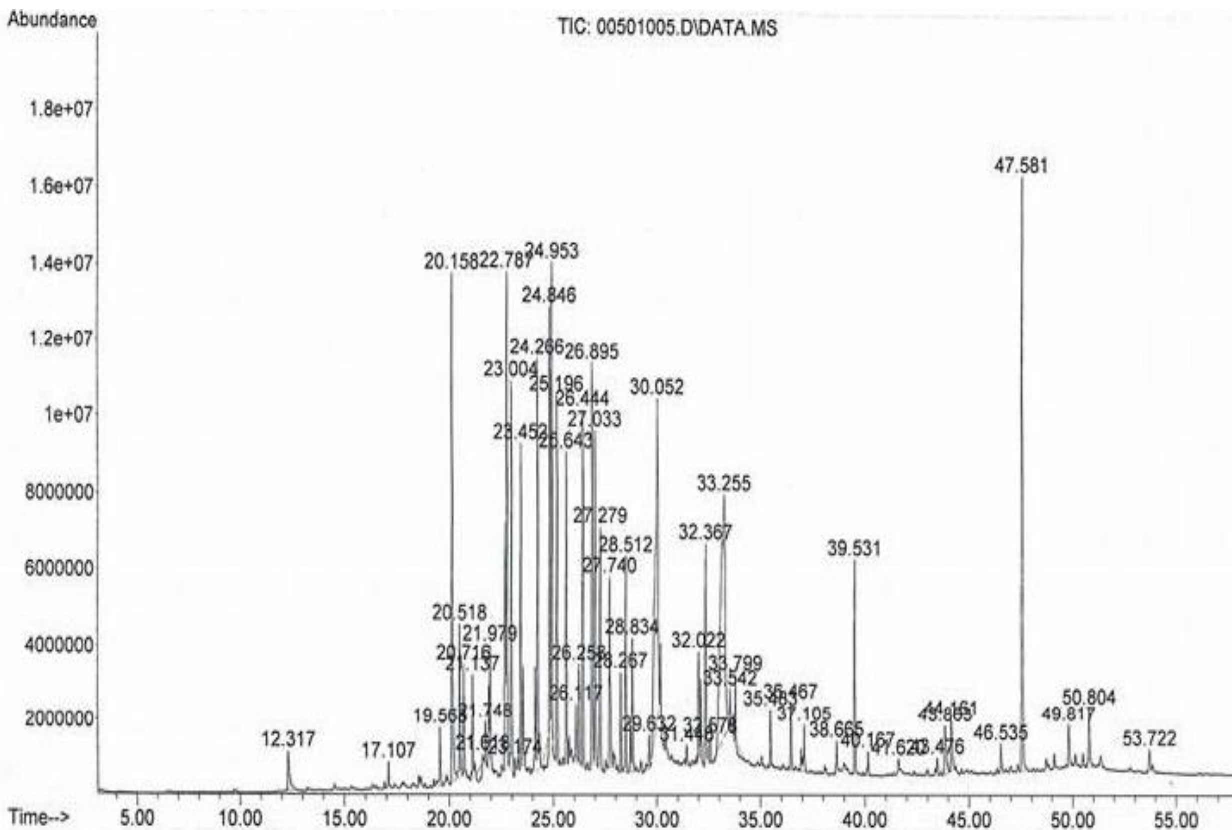

Figure 2. Gas ion chromatogram of the *n*-hexane extract of *P. farcta* aerial part.

and 2-methyl-1-tertiobutilprop-1,3-yl- diisobutylate (5.4%); for leaves part are phytol (39.65%) and benzyl benzoate (6.8%), for flower part are 6,10,14-trimethylpentadecan-2-one (4.8%) and tetracosane (4.4%); and finally for pod part are phytol (15.9%), 3-hydroxy-beta damascone (4.8%) and methyl hexadecanoate (3.1%) (Harzallah-Skhiri et al., 2006).

Antimicrobial activity

The antimicrobial activity of the different solvent extracts, that is, *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol of *P. farcta* was evaluated using disk agar assay against twelve pathogenic microbial strains. The results revealed that the *n*-hexane extract showed moderate antimicrobial activity (relative to the used standard antibiotic) against only three tested strains that is, *Shigella* spp., *E. coli* and *P. vulgaris* with inhibition zones of 4.7, 8.3 and 6.3 mm, respectively; compared to Streptomycin, Ampicillin as standard antibiotics with inhibition zones of 14, 24 and 18 mm respectively. Also, the methylene chloride extract showed moderate antimicrobial activity against three tested strains namely; *Erwinia spp., E. coli* and *S. epidermis* with inhibition zones of 6.2, 7.2 and 8.4 mm respectively; compared to Streptomycin and Ampicillin as standard antibiotics with inhibition zones of 35, 24 mm respectively (Table 3). On the other hand, the ethyl acetate extract exhibited strong antimicrobial effect against four tested strains namely; *Shigella* spp., *E. coli*, *P. vulgaris* and *C. albicans* with inhibition zones of 7.3, 11, 6 and 7.3 mm, respectively; compared to Streptomycin, Ampicillin and Clotrimazole with inhibition zones of 14, 24, 18 and 20 mm, respectively. Moreover, the *n*-butanol extract exhibited strong antimicrobial effect against six tested strains namely; *Shigella* spp., *Erwinia spp., E. coli*, *P. vulgaris*, *S. epidermis* and *C. albicans* with inhibition zones of 11, 9, 17, 12.4, 9.7 and 11 mm, respectively; compared to Streptomycin, Ampicillin and Clotrimazole with inhibition zones 14, 35, 24, 18, 24 and 20 mm respectively (Table 4). The antibacterial activity of the 85% methanol extract of different parts of *P. farcta* growing in Iran against methicillin-resistant *S. aureus* (MRSA) was evaluated with inhibition zones of 5, 6, 8 and 12 mm, respectively for roots, leaf, pods and seeds extracts (Sharifi-Rad et al., 2014). Also, Miri et al. (2015) reported on the antibacterial activity of silver nanoparticles (Ag-NPs) from the crude extract of *P. farcta* growing in Iran against four human pathogenic bacteria namely; *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* with inhibition zone of 9.5, 9, 9.5 and 9.5 mm respectively (Miri et al., 2015). In,

Table 2. The compounds identified in the methylene chloride extract of *P. farcta* by GC/MS analyses.

R.T	Compound Name		Area%	M.F	Molecular ion peak, M^+		Base peak
	No.				m/z	%	
17.85	1	Vanillin	2.84	$C_8H_8O_3$	152	100	151
20.15	2	1,2-dihydrophenanthrene-4-carboxylic acid	5.54	$C_{15}H_{12}O_2$	224	2.97	191
20.51	3	Dihydroactinidiolide	1.24	$C_{11}H_{16}O_2$	180	43.97	111
22.26	4	(1S,4aS,8aS)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylic acid	1.91	$C_{15}H_{24}O_2$	236	2.67	43
22.49	5	3-Hydroxy-β-damascone	6.53	$C_{13}H_{20}O_2$	208	66.34	69
22.69	6	trans-piperitone oxide	5.11	$C_{10}H_{16}O_2$	168	9.35	71
22.90	7	2-Hydroxypinan-3-one	2.78	$C_{10}H_{16}O_2$	168	2.35	71
23.27	8	3a,7a-dimethylhexahydro-4,7-epoxyisobenzofuran-1,3-dione	0.17	$C_{10}H_{12}O_2$	196	1.65	43
23.55	9	Ar-turmerone	1.20	$C_{15}H_{20}O$	216	30.75	83
23.78	10	Benzenemethanol, 3,4,5-trimethoxy-	3.09	$C_{10}H_{14}O_4$	198	100	198
24.21	11	Phenethyl carbamic acid, ethyl ester	3.06	$C_{11}H_{15}NO_2$	193	100	193
24.89	12	Spiro[2,4,5,6,7,7a-hexahydro-2-oxo4,4,7a-trimethylbenzofuran]-7,2'-(oxirane)	1.16	$C_{12}H_{16}O_3$	208	6.29	43
25.65	13	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy	3.48	$C_{10}H_{12}O_3$	180	90.38	137
26.02	14	Loliolide	3.62	$C_{10}H_{16}O_3$	196	10.61	111
26.46	15	Myristicin	1.90	$C_{11}H_{12}O_3$	192	1.96	124
26.63	16	Cedrelanol	1.07	$C_{15}H_{26}O$	222	1.60	43
26.83	17	7-Oxabicyclo[4.1.0]heptan-2-one, 1-methyl-4-(2-methyloxiranyl)	1.95	$C_{10}H_{14}O_3$	182	2.68	111
27.20	18	6,10,14-Trimethylpentadecan-2-one	1.26	$C_{18}H_{36}O$	268	1.61	43
27.77	19	(1R,4S,5R,6R,7S,10R)-7-isopropyl-4,10-dimethyl-tricyclo[4.4.0.0(1,5)]decan-4-ol	0.97	$C_{15}H_{26}O$	222	3.97	149
28.82	20	Hexadecanoic acid, methyl ester	3.91	$C_{17}H_{34}O_2$	270	13.83	74
29.81	21	Tricosanoic acid	9.24	$C_{23}H_{46}O_2$	354	1.64	73
30.06	22	1-Docosene	2.04	$C_{22}H_{44}$	308	1.76	97
32.11	23	11-Octadecenoic acid, methyl ester	2.26	$C_{19}H_{36}O_2$	296	4.37	55
32.33	24	*Cis*-Phytol	2.82	$C_{20}H_{40}O$	296	2.94	71
33.03	25	(Z,Z)-9,12-Octadecadienoic acid	9.10	$C_{18}H_{32}O_2$	280	19.64	67
33.68	26	10-Heneicosene	1.23	$C_{21}H_{42}$	294	2.67	97
34.36	27	1-Heptacosanol	0.88	$C_{27}H_{56}O$	396	1.37	55
35.45	28	Cyclopropaneoctanal,2-octyl	0.51	$C_{19}H_{36}O$	280	2.35	57
36.44	29	2(3H)-Furanone, 5-butyldihydro-4-methyl	1.73	$C_9H_{16}O_2$	156	1.87	99
37.01	30	Cyclotetracosane	1.07	$C_{24}H_{48}$	336	1.96	83
47.55	31	*3β*- Cholest-5-en-3-ol	0.59	$C_{31}H_{50}O_2$	454	2.39	165
49.82	32	D:C-Friedoolean-8-en-29-oic acid	1.08	$C_{30}H_{48}O_3$	456	1.98	207
Total %			**85.34%**				

conclusion these extracts may be used as a source of naturally occurring antimicrobial agents and alternative to the synthetic antibiotics to overcome the microbial infections.

Anticancer activity

The *in vitro* anticancer activity of different solvent extracts of *P. farcta* was evaluated against four human tumor cell lines *viz.*, hepatocellular carcinoma (HePG-2), mammary gland breast cancer (MCF-7), human prostate cancer (PC3) and Epitheliod carcinoma (Hela). According to National Cancer Institute (NCI) criteria, the plant extract with IC_{50} (µg/ml): 1-10 was considered very strong; 11-20 (strong); 21-50 (moderate); 51-100 (weak) and above 100 (non-cytotoxic). Therefore, the ethyl acetate extract showed very strong anticancer activity against Hela and MCF-7 cell lines with IC_{50} of 6.9 and 8.8 µg/ml compared to 5-fluorouracil as standard with IC_{50} of 4.8 and 5.4 µg/ml respectively. On the other hand the *n*-butanol extract showed very strong anticancer activity against MCF-7, HePG-2 and PC3 cell lines with IC_{50} of 5.6, 8.4 and 9.6 µg/ml respectively (Table 5). To the best of our

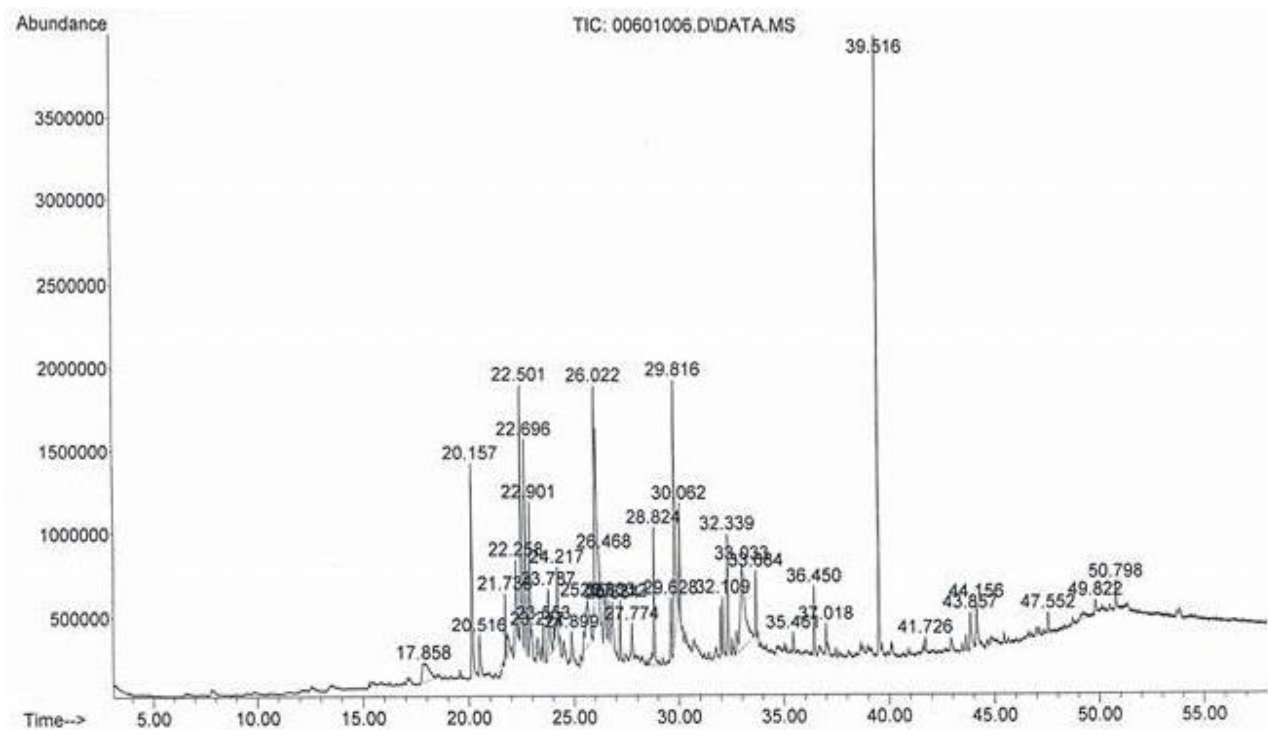

Figure 3. Gas ion chromatogram of the methylene chloride extract of *P. farcta* aerial part.

Table 3. The inhibition zones in mm and activity index% of the *n*-hexane and methylene chloride extracts of *P. farcta* compared to standard antibiotics.

Microorganism	Standard antibiotic/ Inhibition zone (mm)	*n*-hexane		Methylene chloride	
		Inhibition zone (mm)	Activity index%	Inhibition zone (mm)	Activity index%
Klebsiella pneumoniae	Ampicillin/ 25	0	0	0	0
Shigella sp.	Streptomycin/ 14	4.7	33.5	0	0
Erwinia sp.	Streptomycin/ 35	0	0	6.2	17.7
Escherichia coli	Ampicillin/ 24	8.3	34.5	7.2	30
Enterobacter aerogenes	Kanamycin/ 20	0	0	0	0
Pseudomonas aeruginosa	Tobramycin/ 15	0	0	0	0
Proteus vulgaris	Ampicillin/ 18	6.3	35	0	0
Staphylococcus epidermis	Ampicillin/ 24	0	0	8.4	35
Streptococcus pyogenes	Ampicillin/ 20	0	0	0	0
Staphylococcus aureus	Ampicillin/ 24	0	0	0	0
Bacillus subtilis	Kanamycin/ 20	0	0	0	0
Candida albicans	Clotrimazole/ 20	0	0	0	0

knowledge, there is very little information about the anticancer activity reported about the plant. The anticancer activity of the hydroalcoholic extract of *P. farcta* was evaluated against MKN45 (human gastric cancer) cell line (Dejamfekr and Khaleghian, 2016). The above mentioned results suggest that *P. farcta* plant may be used as a source of naturally occurring anticancer agents.

Free radical scavenging antioxidant activity (ABTS assay)

The free radical antioxidant activity of different solvent extracts of *P. farcta* was evaluated via ABTS assay which based on their abilities to reduce the radical cation $ABTS^{+\circ}$. The results in (Table 6) revealed that the % inhibition of ABTS radical was 83.1, 82.0, 87.2 and 87.0%

Table 4. The inhibition zones in mm and activity index% of the ethyl acetate and *n*-butanol extracts of *P. farcta* compared to standard antibiotics.

Microorganism	Standard antibiotic/ inhibition zone (mm)	Ethyl acetate		*n*-butanol	
		Inhibition zone (mm)	Activity index%	Inhibition zone (mm)	Activity index%
Klebsiella pneumoniae	Ampicillin/ 25	0	0	0	0
Shigella spp.	Streptomycin/ 14	7.3	52.1	11	78.5
Erwinia spp.	Streptomycin/ 35	0	0	9	25.7
Escherichia coli	Ampicillin/ 24	11	45.8	17	70.8
Enterobacter aerogenes	Kanamycin/ 20	0	0	0	0
Pseudomonas aeruginosa	Tobramycin/ 15	0	0	0	0
Proteus vulgaris	Ampicillin/ 18	6	33.3	12.4	68.8
Staphylococcus epidermis	Ampicillin/ 24	0	0	9.7	40.4
Streptococcus pyogenes	Ampicillin/ 20	0	0	0	0
Staphylococcus aureus	Ampicillin/ 24	0	0	0	0
Bacillus subtilis	Kanamycin/ 20	0	0	0	0
Candida albicans	Clotrimazole/ 20	7.3	52.1	11	78.5

Table 5. *In vitro* anticancer activity of different solvent extracts of *P. farcta* against four human tumor cell lines compared to 5-fluorouracil as standard.

Sample	IC_{50} (μg/ml)[1]			
	HePG-2	Hela	PC3	MCF-7
5-FU[2]	7.9±0.28	4.8±0.21	8.3±0.35	5.4±0.20
n-hexane	36.2±2.32	51.5±2.98	40.4±2.35	33.9±2.74
Methylene chloride	62.4±3.74	67.1±3.81	41.2±2.28	57.7±3.35
Ethyl acetate	14.6±1.06	6.9±0.36	12.4±1.24	8.8±0.86
n-butanol	8.4±0.41	13.5±0.98	9.6±0.87	5.6±0.53

[1]IC_{50} (μg/ml): 1-10 (very strong); 11-20 (strong); 21-50 (moderate); 51-100 (weak) and above 100 (non-cytotoxic).[2]**5-FU**: 5-fluorouracil.

Table 6. *In vitro* ABTS free radical antioxidant activity of different solvent extracts of *P. farcta* compared to ascorbic acid.

Sample	ABTS Abs(control)-Abs(test)/Abs(control) × 100	
	Absorbance of samples	% inhibition
Ascorbic acid	0.055	89.2
n-hexane	0.086	83.1
Methylene chloride	0.092	82.0
Ethyl acetate	0.065	87.2
n-butanol	0.066	87.0

respectively for the *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol extracts compared to ascorbic acid as standard (89.2%). The IC_{50} value of the ABTS free radical activity of the methanol extract of *P. farcta* fruits growing in Tunisia was 270 μg/ml (Lajnef et al., 2015). To the best of our knowledge, there are limited reports about the antioxidant activity of *P. farcta* using the ABTS assay, but there are available data about other assays that is, the antioxidant activity of four solvent extracts from pod and seed of *P. farcta* was evaluated using DPPH assay, and the IC_{50} of the tested extracts from pod part were 1.0, 6.15, 3.55 and 7.75 μg/ml respectively for ethanol, methanol, octanol and *n*-heptane extracts, while and the IC_{50} of the tested extracts from seed part were 2.0, 1.51, 0.95 and 4.45 μg/ml respectively for ethanol, methanol, octanol and *n*-heptane extracts (Poudineh et al., 2015).

The antioxidant activity of plant extracts as a complex mixture may be attributed to the presence of polyphenolic compounds due to therir abilities to act as free radicals scavengers either in individual mode or via synergetic mode (co-activity) (Ivanova et al., 2005; Djeridane et al., 2006).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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