INTRODUCTION

Cancer, after cardiovascular disease, is the second main cause of death [1]. Treatment usually comprised of various combinations of surgery, chemotherapy and radiation therapy, but regardless of these therapeutic options, cancer remains associated with high mortality. Natural and some synthetic compounds can prevent, curb, or reverse the headway of cancer. The enforcement and research for drugs obtained from plants have increased in recent years, while herbs and their constituents are generally known to be safe, either because of their traditional use without any legalized perilous impact or because of reported toxicological studies [2]. Presently, there has been an increased advantage globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in prophylactic medicine. The use of traditional medicine is widespread in Africa, and medicinal plants are still a senior source of natural antioxidants that might do as leads for the evolution of novel drug against free radical induced diseases. Abutilon [3] is a large genus of flowering plants in the mallow family, Malvaceae. It is distributed throughout the tropics and subtropics of the Americas, Africa, Asia, and Australia [4]. Various plants of Abutilon species are traditionally assumed for their varied pharmacological and medicinal properties and treatment of various ailments [5]. Furthermore, different plant parts contain specific phytoconstituents responsible for their biological activities. The flowers and leaves of some Abutilon species are applied as antiasthmatic and anti-inflammatory for urethritis, boils, and ulcers [6]. Several Abutilon species have been reported to consist of several constituents such as triterpenes, sterols [7], sesquiterpene lactones [8], flavonoids [9], phenolic acids [10], and essential oil [11]. The leaf extract of Abutilon indicum has been reported for the hepatoprotective activity [12]. Moreover, it is used to treat several disorders including diabetes mellitus [13]. Abutilon theophrasti Medik. has been demonstrated to have numerous pharmacological activities [14], such as expelling wind, detodification, and anti-inflammatory activities; it is primarily used for otitis media, rheumatic pains, bruises, arthralgia, sprains, tinnitus, dysentry, and deafness treatments.

Abutilon hirtum (Lam.) sweet is a perennial herb, growing in Tropical Africa, Asia and Australia, naturalized in India, South Florida, Mexico and South America [15]. In a previous publication, the lipid and mucilage contents of A. hirtum leaves possesses cytotoxic activity [16]. The seeds are useful in expellantur, piles, laxative, in chronic cystitis, gonorrea, and gleet [17-19]. A. hirtum has been taken up to give scientific evidence to the folklore demand on the hepatoprotective activity of the leaves in the form of decoction [20]. Decoction of the leaves used as mouth wash, bladder inflammations, wounds, and treatment of ulcers [21]. Alkaloids are reported from the roots of the plant [22]. Among this, the literature describes the presence of cholestan derivative in Abutilon bidentatum [23]. Secondary metabolites such as phenols, tannins, flavonoids [24], and steroids have been reported to possess antimicrobial, antioxidant and anticancer [25,26] properties in many studies.

The objective of our present investigation was to determine the cytotoxicity, antimicrobial, and antioxidant properties of some compounds isolated from A. hirtum by carrying out various experimental studies.

MATERIALS AND METHODS

Plant materials

A. hirtum (Lam.), Malvaceae, leaves were collected at May from El-Zohria garden, Cairo, Egypt. The plant was authenticated by Wafaa M. Amer, Professor of Taxonomy, Department of Botany, Faculty of Science, Cairo University, Giza, Egypt. Voucher specimens (Reg. No.: A1) were deposited at the herbarium, Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.
General experimental procedures

Melting points were determined using a Melt-Apparatus (SMDP3 Stuart Scientific UK). Gas chromatography-mass spectrometry (GC-MS) data were determined on an electrospray ionization MS (ESI-MS) was measured on a Finnigan TSQ 700 GC/MS coupled with a Finnigan electrospray source. $^1$H NMR (300 MHz) and $^{13}$C NMR (75 MHz) data were obtained on Jeol GLM spectrometers relative to tetra methyl silane in dimethyl sulfoxide (DMSO)-d$_6$. The absorbance measurements for antioxidant activity were recorded using the ultraviolet and visible (UV-vis) spectrophotometer Spectronic 601 (Milton Roy, USA). For column chromatography, silica gel (70-230 mesh) (Merck), Sephadex LH 20 (Pharmacia, Uppsala, Sweden), and polyamide 6S (Riedel de Darmstadt, Germany) were used. Paper chromatography (PC) was done on Whatman No. 1 (57×46 cm), while thin layer chromatography (TLC) was carried out on readymade silica plates (GF$_2$54, Merck) and visualized under UV light. The microplate reader (SunRtek, TECAN, Inc., USA) was used to determine the number of viable cells and the percentage of viability. The solvents and reagents used herein were of analytical grade. Aluminum chloride, ferric chloride, sodium bicarbonate, sodium phosphate, ascorbic acid, and ammonium molybdate were purchased from Merck Chemical Co., all solvents and acids (petroleum ether, chloroform, ethyl acetate, methanol, n-butanol, sulfuric acid, acetic acid, aniline phthalate, pyridine-d$_5$, benzene, and DMSO) were purchased from Sigma-Aldrich Co. Human hepatocellular carcinoma (HepG2) cell line was obtained from the American type culture collection (ATCC, Rockville, MD). Fetal calf serum (FCS) (Hyclone, Logan, Utah, USA). Roswell Park Memorial Institute (RPMI), 1640 medium (Sigma, Chem. Co, St Louis, MO, USA).

Equipment and chemicals for antimicrobial assays

Nutrient agar medium (LAB M, UK), NaNO$_3$ (S. D. Fine-Chem. Ltd.), sucrose (Oxford), MgSO$_4$ (S. D. Fine Chem. Ltd.), FeSO$_4$ (S. D. Fine Chem. Ltd.), KCl (S. D. Fine Chem. Ltd.), K$_2$HPO$_4$ (MERCK), agar-agar bacto (S. D. Fine Chem. Ltd.), Staphylococcus aureus (G +ve bacteria) (ATCC 6538-P), Candida albicans (yeast) (ATCC 27853), Pseudomonas aeruginosa (G -ve bacteria) (ATCC 10231), and Aspergillus niger (fungi) (NRRLA-326). Low temperature incubator SHEL-LAB model 2005 shield on manufacturing, Inc. NUAJARE Biological safety cabinet, LABSCO oven Laboratory Supply Company, Autoclave la Astell Heerlon Germany, Almon and Co. KG Germany, Refrigerator Toshiba (no frost model FR-GF40P). All the test microbes were obtained from the culture collection from Microbial Chemistry Department, National Research Center.

Solvent systems

n-BuOH:AcOEt:H$_2$O (4:1:5, top phase) (PC), (S1) AcOH:H$_2$O (15:85) (PC), S2; EtOAc:MeOH:H$_2$O (15:10:2) (TLC), S3; CHCl$_3$:MeOH:H$_2$O (8:2.0.3) (TLC), S4; CH$_3$COCH$_3$:MeOH 8:2 (TLC) SS and CH$_3$COCH$_3$:MeOH 9:5.0.5 (TLC) S6.

Statistical analysis

All results were given as the mean ± standard deviation of three replicates using SPSS v 13.0 program. The analysis of all experiments was analyzed by Microsoft Excel program for statistical significance.

Evaluation of total antioxidant capacity (TAC)

The TAC of each sample was determined according to phosphomolybdenuem method according to Prieto et al., 1999, using ascorbic acid as a standard. In this method, 0.5 ml of each sample (200 µg/ml) in methanol mixed in dried tubes with 5 ml of standard reagent solution containing (0.6 M sulfuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate). The tubes containing the reaction solution mixture were capped and incubated in a thermal block at 95°C for 90 minutes. The samples had cooled at room temperature; then, the absorbance was measured at 695 nm using a UV-vis spectrophotometer Spectronic 601 against the blank. The blank consisted of all reagents solvents and without the sample and it was incubated under the same conditions. All experiments were carried out in triplicate. The antioxidant activity of the compounds was expressed as the number of ascorbic acid equivalents (AAE) [27].

Cytotoxic activity assay of liver carcinoma cell line (HepG2)

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum FCS and 50 µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO$_2$ and were subcultured two to three times a week. For antitumor assays, the tumor cell lines were suspended in medium at concentration 5×10$^4$ cell/well in Corning® 96-well tissue culture plates and then incubated for 24 hrs. The tested compounds were then added into 96-well plates (six replicates) to achieve eight concentrations for each compound. Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubating for 24 hrs, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plates and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 ml of phosphate buffered saline) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO$_2$ for 4 hrs. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 minutes. Then, the optical density was measured at 590 nm with the microplate reader to determine the number of viable cells and the percentage of viability was calculated as $\left[1 - \frac{\text{ODt}}{\text{ODc}} \right] \times 100\%$, where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC$_{50}$) the concentration required to cause toxic effects in 50% of intact cells was estimated from graphic plots of the dose response curve for each conc. using GraphPad Prism software (San Diego, CA, USA) [28].

Antimicrobial activity bioassay

Disc agar plate method was done to evaluate the antimicrobial activity of methanol extract from A. hirtum plant and the isolated pure compounds. The antimicrobial activities of 0.5-cm-diameter filter paper disc saturated with about 1 mg sample were tested against four different microbial strains, i.e., S. aureus (G +ve bacteria), P. aeruginosa (G -ve bacteria), C. albicans (yeast), and A. niger (fungi). Both bacterial and yeast test microbes were grown on nutrient agar (DSNZ 1) medium (g/L); Beef extract (3), agar (20) and peptone (10), whereas fungal test microbe was grown on Sazpek-Dox (DSMZ 130) medium (g/L); Sucrose (30), MgSO$_4$.7H$_2$O (0.5), NaNO$_3$ (3), KCl (0.5), FeSO$_4$.7H$_2$O (0.001), K$_2$HPO$_4$ (1), and agar (20). The culture of each microorganism was diluted by sterile distilled water to 10$^{-3}$ to 10$^{-6}$ CFU/ml to be used as inoculum. 1 ml of the previous inoculum was used to inoculate 11 of agar medium (just before solidification) then poured in Petri dishes (10 cm diameter containing 25 ml). Discs (5 mm diameter) were placed on the surface of the agar plates previously inoculated with the test microbe and incubated for 24 hrs for bacteria and yeast but for 48 hrs for fungus at 37°C and 30°C respectively [29].

Acid hydrolysis of compounds 2, 7, 8 and 9

A solution of each compound (5 mg) was hydrolyzed in aqueous solution of 2M HCl (5 ml) was heated in a water bath at 100°C for 2 hrs. The solution mixture was evaporated in a rotary evaporator in vacuum to dryness and was dissolved in MeOH, then the mixture was partitioned between chloroform and water using separating funnel. The chloroform extract was evaporated under reduced pressure then crystallized to give the aglycone. The sugar was extracted with pyridine from the aqueous layer after neutralized with NaHCO$_3$, then filtered and concentrated. The aglycone was detected on PC using CO-PC with authentic aglycone samples and visualized under UV and with NH$_3$ vapor. Sugar moieties were detected on silica gel TLC plates with EtOAC:MeOH:H$_2$O:AcOH (13:3:3:4) using aniline phthalate in n-butanol (freshly prepared), as a spraying reagent. Furthermore, further confirmation for the sugar was done on PC (Whatmann paper No. 1), via solvent system (n-butanol:pyridine-d$_5$:water [10:3:3]), then the sugar spots were detected by spraying with freshly prepared aniline phthalate sugar reagent.
Extraction and isolation
Dried-powdered leaves (1600 g) were boiled under reflux in 10 L of petroleum ether for 8 hrs. After filtration, the solvent was evaporated; afforded 15 g of petroleum ether extract and the leaf powder dried, then boiled with reflux for 8 hrs with chloriform, then filtration and the residue was evaporated to give 34 g chloroform extract. Ethyl acetate was added and refluxed for 8 hrs, then filtration and evaporation to give 31 g ethyl acetate extract. Finally, the leaves were refluxed with 10 L of 85% aqueous MeOH, after cooling, the solution was filtered and evaporated, and the residue was dissolved in de-ionized water (250 ml), then the salt was removed by adding excess methanol solution (2.5 L), and finally filtered. The filtrate was dried to give (96 g) extract, which was chromatographed on polyamide column chromatography GC (120×7 cm, 355 g) eluted successively with 0, 25, 50, 75, and 100% MeOH in H$_2$O, each 1 L to give five fractions (A: 13.5 g, B: 3.73 g, C: 5.7 g, D: 4.0 g, and E: 1.4 g, respectively). Fraction A was applied to a silica gel CC eluted with a gradient of MeOH:H$_2$O (15:100) to afford 30 subfraction (each 20 ml). Sub fraction [6-14] (219 mg) eluted with CHCl$_3$:MeOH (5:5:5:5) on silica gel GC, were further separated by Sephadex LH-20 CC eluted with a gradient of MeOH:H$_2$O (from 2.8 to 10:0) to yield 28 mg compound of 7. Subfraction [15-30] (257 mg) eluted with CHCl$_3$:MeOH (5:5), were further separated by Sephadex LH-20 CC eluted with a gradient of MeOH:H$_2$O (from 3.7 to 10:0) to yield compound 6 (54 mg). Fraction B eluted with CHCl$_3$:MeOH:H$_2$O (7:3:0-7:3:5), was further separated on silica gel column chromatography over successively eluted with CHCl$_3$ and CHCl$_3$:MeOH with increasing contents of methanol afforded compound 8 (45 mg) and 9 (32 mg). Fraction E was subjected to CC over silica gel using gradient elution with CH$_3$CO$_2$:H$_2$O (10:0-3:7) afforded 24 subfraction (each 15 ml) possess mixture of compounds; subfraction [2-9] subjected to preparative silica gel chromatography afforded compound 3 (27 mg), then subfractions [10-24] was re-purified on Sephadex LH-20 CC and eluted with MeOH:H$_2$O (9.5:0.5) affording compound 10 (36 mg).

RESULTS AND DISCUSSION
Column chromatographic separation of the 85% methanol extract of $A$. hirtum leaves led to the isolation of ten compounds, nine of them are phenolics, the structure of the isolated compounds were established by spectroscopic analysis. The isolated compounds identified as methyl gallate, cuneataside E, bergapten, gallic acid, ellagic acid, phenolics A. hirtum, and it showed a single signal in aromatic region in $H$ NMR spectrum at δ 7.6 (2H, s, H-2 and H-6). $C$ NMR data showed five signals in aromatic region identical to that of gallic acid, δ 168.2 (C=O), 145.7 (C-3, 5), 138.5 (C-4), 121.8 (C-1) and 109.6 (C-2, 6). Gallic acid is widely distributed in tannin containing plants [33].

Compound 2 (kaempferol-3-O-α-L-rhamnoside): It is obtained as yellow powder, m.p. 278°C, R$_f$: 0.70 [52], $ESI$-MS mass spectra gave a molecular ion at m/z 495 (M+H$^+$_); $′H$ NMR showed signals at δ 7.7 (4H, d, J=2.0 Hz, H-2 and H-6) respectively. Fraction A was applied to a silica gel CC eluted with a gradient of MeOH:H$_2$O (7:7:2:2), $′H$ NMR showed signals at δ 7.6 (2H, d, J=2.0 Hz, H-2 and H-6) respectively. It showed similarity with methyl gallate spectrum data and it showed similarity with methyl gallate spectrum data (37 mg).

Compound 3 (kaempferol-3-O-α-L-rhamnoside): It is obtained as yellow powder, m.p. 278°C, R$_f$: 0.70 [52], $ESI$-MS mass spectra gave a molecular ion at m/z 495 (M+H$^+$_); $′H$ NMR showed signals at δ 7.7 (4H, d, J=2.0 Hz, H-2 and H-6) respectively. Fraction A was applied to a silica gel CC eluted with a gradient of MeOH:H$_2$O (7:7:2:2), $′H$ NMR showed signals at δ 7.6 (2H, d, J=2.0 Hz, H-2 and H-6) respectively. Compound 3 showed signals characteristic of a furanocoumarin bergapten which previously isolated from Portulaca oleracea [32].

Compound 4 (gallic acid): It is obtained as buff needles, m.p. 255°C, R$_f$: 0.49 [52], it showed similarity with methyl gallate spectrum data where $ESI$-MS mass spectra give a molecular ion at m/z 303 (M+H$^+$) and it showed a single signal in aromatic region in $H$ NMR spectrum at δ 7.6 (2H, s, H-2 and H-6). $C$ NMR data showed five signals in aromatic region identical to that of gallic acid, δ 168.2 (C=O), 145.7 (C-3, 5), 138.5 (C-4), 121.8 (C-1 and C-2, 6). Gallic acid is widely distributed in tannin containing plants [33].

Compound 5 (elagic acid): It is obtained as gray crystals, m.p. 353°C, R$_f$: 0.67 [52], $ESI$-MS mass spectra gave a molecular ion at m/z 303 (M+H$^+$) and it showed a single signal in aromatic region in $H$ NMR spectrum at δ 7.6 (2H, s, aromatic-H). It gives blush green color with ferric chloride. According to mixed m.p. and color reaction on PC comparable with authentic sample; compound 5 identified as elagic acid [34].

Compound 6 (EGGC): It is isolated as cream powder m.p. 212°C, R$_f$: 0.26 [51], 0.74 [52], $ESI$-MS mass spectra gave a molecular ion at m/z 459 (M+H$^+$); $′H$ NMR showed signals at δ 7.7 (4H, d, J=2.0 Hz, H-2 and H-6); δ 5.6 (1H, d, J=2.0 Hz, H-6), δ 5.6 (1H, d, J=2.0 Hz, H-6), δ 5.2 (1H, m, H-3), δ 4.90 (1H, d, J=5.6 Hz, H-2), δ 1.99 (2H, m, H-4), these data confirmed by $^{13}$C NMR data (Table 1) that suggested compound 6 was EGGC [33].

Compound 7 (kaempferol-3-O-β-D-glucopyranoside): It is isolated as yellow powder, m.p. 278°C, R$_f$: 0.70 [52], $ESI$-MS mass spectra gave a molecular ion at m/z 400 (M+H$^+$); $′H$ NMR showed signals in aromatic region similar to that of kaempferol at δ 2.86 (2H, d, J=8.0 Hz, H-2 and H-6), δ 8.69 (2H, d, J=8.0 Hz, H-2 and H-6), δ 6.65 (1H, d, J=2.5 Hz, H-8), δ 6.40 (1H, d, J=2.5 Hz, H-6) in addition to signal at δ 4.95 (1H, brs, anomeric proton). $′C$ NMR data showed signals characteristic of rhamnose. Acid hydrolysis revealed the presence of rhamnose in the aqueous portion. $^{13}$C NMR data agreed with the previous data and suggested that compound 7 identified as kaempferol-3-O-β-D-glucopyranoside which previously isolated from other plants [33,35].

Compound 8 (benzyl-1-O-β-D-glucopyranoside): It is isolated as white needles, m.p. 210-215°C, R$_f$: 0.86 [51], $ESI$-MS mass spectra gave a molecular ion at m/z 271 (M+H$^+$); $′H$ NMR showed multiplet signal at δ 7.70-7.67 (5H, m, H-2, 3, 4, 5, 6), characteristic for mono-substituted aromatic ring. It also showed many signals at δ 6.14 (1H, d, J=1.18 Hz, H-7a), 6.50 (1H, d, J=11.8 Hz, H-7b) and 4.0-3.37 (six glucose protons) $^{13}$C NMR spectrum (Table 1); showed 13 signals, two oxymethylenes at δ 57.05 (CH$_3$O), and 60.30 (CH$_2$OH, glu), five oxymethines (CH$_2$OH, glu), five methane (aromatic CH) and one quaternary carbon (aromatic C$_6$). Acid hydrolysis suggests the presence of mono-substituted aromatic ring and hexose sugar.

Complete acid hydrolysis indicates that the sugar was glucose; these data suggested that compound 8 is benzyl-1-O-β-D-glucopyranoside; the compound was previously isolated from Aeverhoa carambola L. [36].
Apoptosis is a controlled cell death mechanism functional under both normal and pathological conditions during which cells that are damaged or redundant are eliminated within the body. This is particularly important during embryogenesis and early developmental stages when a large number of immature neuronal cells are eliminated during formation and maturation of the nervous system [40]. The results suggested that plant phenol, gallic acid, and methyl gallate, may play an important role in the cytotoxic activity of the plant. Bergapten is known to cause chromosome abnormality, while some plant compounds have anti-apoptotic properties [25,39].

### Table 1: $^{13}C$ NMR of compounds 2, 3, 6, 7, and 9 (300, 75 MHz, DMSO-d$_6$)

| Carbon number | Compound 2 | Compound 3 | Compound 6 | Compound 7 | Compound 8 | Compound 9 |
|---------------|------------|------------|------------|------------|------------|------------|
| 1             | 40.1       | -          | 72.2       | 156.6      | 127.0      | 116.7      |
| 2             | 46.9       | 161.2      | 90.3       | 185.7      | 120.3      | 117.4      |
| 3             | 76.0       | 17.7       | 150.0      | 162.0      | 107.0      | 111.7      |
| 4             | 40.4       | 18.7       | 150.0      | 156.2      | 120.0      | 116.7      |
| 5             | 127.4      | 11.2       | 94.3       | 99.0       | 125.7      | 131.1      |
| 6             | 138.2      | 112.9      | 156.4      | 165.4      | 70.5       | 37.0       |
| 7             | 24.0       | 159.6      | 156.9      | 165.4      | 70.5       | 37.0       |
| 8             | 46.5       | 92.7       | 156.9      | 165.4      | 70.5       | 37.0       |
| 9             | 220.0      | 152.6      | 156.4      | 165.4      | 70.5       | 37.0       |
| 10            | 31.9       | 106.7      | 95.7       | 101.7      | 101.7      | 101.7      |
| 11            | 30.1       |            |            |            |            |            |
| 12            | 31.3       |            |            |            |            |            |
| 13            | 21.5       |            |            |            |            |            |

$\delta$ in ppm

### Table 2: $^{13}C$ NMR of compound 10 (300, 75 MHz, DMSO-d$_6$)

| Carbon no | $\delta$ |
|-----------|----------|
| 1         | 37.3     |
| 2         | 32.0     |
| 3         | 75.2     |
| 4         | 42.4     |
| 5         | 144.6    |
| 6         | 122.4    |
| 7         | 33.1     |
| 8         | 35.6     |
| 9         | 50.7     |
| 10        | 36.8     |
| 11        | 22.4     |
| 12        | 40.3     |
| 13        | 42.6     |
| 14        | 56.6     |
| 15        | 25.7     |
| 16        | 28.5     |
| 17        | 56.1     |
| 18        | 12.6     |
| 19        | 20.2     |
| 20        | 40.1     |
| 21        | 21.5     |
| 22        | 35.6     |
| 23        | 29.7     |
| 24        | 50.3     |
| 25        | 26.0     |
| 26        | 19.2     |
| 27        | 19.8     |
| 28        | 23.3     |
| 29        | 11.9     |

$\delta$ in ppm
The antimicrobial activities noticed among the methanol extract and isolated compounds of *A. hirtum* leaves were found to be maximum in ellagic acid against (G-ve, G+ve) bacteria, yeast and fungi with inhibition zones ranged from 8 to 14 mm. Gallic acid showed a characteristic effect against *A. niger* with inhibition zone (10 mm). On the other hand, the methanolic extract of *A. hirtum* showed a strong activity against *S. aureus* (15 mm) followed by activity against *C. albicans* (9 mm) and against *P. aeruginosa* (8 mm). In this study, chloroform extract exhibited activity against *P. aeruginosa* and *S. aureus* with inhibition zones 6 mm and 10 mm, respectively. Only compounds 4, 5 and 7 exhibited antifungal activity against *A. niger* (Table 3).

Methyl gallate exhibited strong antimicrobial activity; it is known to possess growth-inhibiting activity against *Escherichia coli*, without adversely affecting the growth of lactic acid-producing bacteria, with the activity being more pronounced by the presence of methyl gallate [42]. Antibacterial activity of some plant extracts was interpreted on the basis of the presence of some polyphenols; many studies were referred to that the activities of medicinal plant extracts can be explained by the presence of tannins, flavonoids, polyphenols, saponins and steroids [43].

Fig. 2: The cytotoxic activity of compounds 2 and 3 against HepG2 cancer cell lines. 4, 5, 6, 8 and 9 against HepG2 cancer cell lines damage and mutagenicity probably due to cross-linkage with DNA, the same mechanism observed for the therapeutic action [41].

**Fig. 1: Chemical skeletons of compounds 1-10**
As in tannin, ellagic acid may induce complexation with enzyme or substrate in bacteria cell. Ellagic acid toxicity may be related to its action on the microorganism membranes. Moreover, the ability of ellagic acid to form complexes with the essential metals in bacteria cell account for its toxicity [44].

Polyphenols are known to form with proteins soluble complexes of high molecular weight. Thus, after being adsorbed, the polyphenols will react with the protein moiety of cell enzymes (oxidoreductases) in the cytoplasm and in the cell wall. They may also bind to bacterial adhesions, and so interfering with the availability of receptors on the cell surface [45].

Tannin with gallate group has various physiological functions such as antibacterial, antiallergic, scavenging free radicals, lowering blood pressure, serum and hepatic cholesterol concentrations and increasing fecal sterol excretion in rats with hypercholesterolemia [46]. EGCG is the most abundant, potent polyphenol and is responsible for most of therapeutic benefits (either clinical, animal or cell culture studies). It has various medicinal potentialities which include antimicrobial properties against resistant microorganisms on which it acts by either disrupting the cell membrane, inhibiting the biosynthesis of the cell constituents, cells signaling or DNA damage (described in following sections) [47].

The methanol and chloroform extracts, as well as the pure compounds, found active could be useful for the development of a new antimicrobial drug. However, pharmacological and toxicity studies currently going on in the laboratory capable of confirm this hypothesis.

**Antioxidant assessment**

The TAC is a quantitative method for determining the TAC, which is expressed as AAEs. This method based on the reduction of Mo(VI) to Mo(V) by the sample and subsequent formation of green phosphate/Mo(V) complex at acid pH. It assesses both water-soluble and fat-soluble antioxidants (TAC). The results indicate higher TAC of the methanol, chloroform extracts and pure compounds, respectively, at low concentration, this method gives a combined measure of the antioxidant activity of the range of chemically various phenolic and flavonoid compounds present in the methanol extract of *A. hirtum* leaves as specified by formation of the reduced phosphomolybdate complex and measured at (695 nm) as indicated in Table 4. Under the experimental conditions, all selected samples exhibited antioxidant activities. Furthermore, a significant correlation between the results of these tests was obtained. These results strongly correlate with the total phenolic and flavonoid contents of the samples, and according to the obtained results, its phenolics and flavonoids are the major phytochemicals responsible for antioxidant activity in the investigated samples. The TAC of *A. hirtum* leaves ranges from 810.35-341.15% of standard ascorbic acid at a concentration of 100 µg/ml of the sample in methanol (Table 4). Accordingly, it is clear that the isolated compounds have high redox potentials and can act as reducing agent, hydrogen donor, and singlet oxygen quencher. Salidroside, a phenylpropanoid glycoside, shows potent antioxidant property which may due to it have interfered in the intracellular excess ROS thereby down-regulated the ROS signaling pathway. Hu's works showed that salidroside induced apoptosis in cancer cells and the cytotoxic effects of salidroside were very strong which may be important for the anticancer activity observed in liver cancer cells [48]. In addition to being used as medicinal herbs, this plant could also be used as a rich source of natural antioxidants to delay the oxidative deterioration of foods. It is also suggested that

### Table 3: Antimicrobial activity of the methanol extract from *Abutilon hirtum* and its purified compounds

| S. No. | Sample                     | Pseudomonas aeruginosa* | Staphylococcus aureus** | Candida albicans*** | Aspergillus niger fungi |
|--------|----------------------------|-------------------------|-------------------------|---------------------|-------------------------|
| Control | Methanol                  | 0                       | 0                       | 0                   | 0                       |
| Crude extract | Methanol extract | 8                       | 15                      | 9                   | 0                       |
| Crude extract | Chloroform extract | 6                       | 10                      | 0                   | 0                       |
| 1       | Compound 1                 | 7                       | 8                       | 5                   | 0                       |
| 2       | Compound 2                 | 9                       | 7                       | 6                   | 0                       |
| 3       | Compound 3                 | 2                       | 3                       |                      | 0                       |
| 4       | Compound 4                 | 10                      | 9                       | 5                   | 10                      |
| 5       | Compound 5                 | 12                      | 14                      | 10                  | 8                       |
| 6       | Compound 6                 | 10                      | 11                      | 10                  | 0                       |
| 7       | Compound 7                 | 7                       | 10                      | 5                   | 8                       |
| 8       | Compound 8                 | 9                       | 4                       | 6                   | 0                       |
| 9       | Compound 9                 | 7                       | 5                       | 0                   | 0                       |
| 10      | Compound 10                | 9                       | 11                      | 8                   | 0                       |

©Control, *(G-ve), **(G+ve), ***Yeast

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![Fig. 3: The cytotoxic activity of compounds. 2 and 3 against HepG2 cancer cell lines](image1.png)

![Fig. 4: The cytotoxic activity of compounds. 1, 7 and 10 against HepG2 cancer cell lines](image2.png)

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As in tannin, ellagic acid may induce complexation with enzyme or substrate in bacteria cell. Ellagic acid toxicity may be related to its action on the microorganism membranes. Moreover, the ability of ellagic acid to form complexes with the essential metals in bacteria cell account for its toxicity [44].

Polyphenols are known to form with proteins soluble complexes of high molecular weight. Thus, after being adsorbed, the polyphenols will react with the protein moiety of cell enzymes (oxidoreductases) in the cytoplasm and in the cell wall. They may also bind to bacterial adhesions, and so interfering with the availability of receptors on the cell surface [45].
Table 4: The T AC using Ascorbic acid as standard

| Sample           | TAC (mg AAE/g sample) |
|------------------|-----------------------|
| Methanol extract | 497.3±1.15            |
| Chloroform extract | 442.0±2.00         |
| Compound 1       | 695.0±1.10            |
| Compound 2       | 341.5±1.75            |
| Compound 3       | 568.4±1.65            |
| Compound 4       | 743.3±0.15            |
| Compound 5       | 810.3±1.55            |
| Compound 6       | 677.7±1.20            |
| Compound 7       | 362.2±1.95            |
| Compound 8       | 450.1±2.00            |
| Compound 9       | 782.1±2.10            |
| Compound 10      | 587.7±2.25            |

Results are expressed as mean value±standard deviation (n=3). *Total antioxidant capacity values are expressed as mg ascorbic acid equivalent/g extract (mg AAE/g sample).

β-sitosterol can protect against oxidative stress through modulation in the levels of antioxidant enzymes [49]. Fatima et al., in an in vitro study revealed that, due to its antioxidant property, EGG is an inhibitor of RDS and reactive nitrogen intermediates pathways [50]. Aerobic organisms consume a large amount of molecular oxygen to maintain cellular metabolic processes. Reactive oxygen species (ROS) are the section of various metabolic processes for which the terminal electron acceptor is the molecular oxygen (O2) that acts as a thermodynamic basin. ROS generally causes harm effect to living organisms and thus, the induced oxidative stress due to the formation of ROS is attributed to the damage of biological systems in the body, supporting the development of various diseases such as cancer and also forced the aging process [51]. Patra et al. (2010) confirmed that β-sitosterol reduced carcinogen-induce cancer of the colon. It also shows anti-inflammatory, antipyretic, antiarthritic, antiulcer, insulin releasing, and estrogenic lowering property [38]. Gallic acid is such a polyphenolic compound with reported antioxidant activities on different cancer cells. It is also well known for its protective activity on normal cells which made gallic acid as a necessary compound for cancer therapy [52]. The impact of the study will pave a way to develop gallic acid as a significant therapeutic agent to treat and prevent cancer. Ellagic acid is a potent antioxidant, a phenolic compound known as a potent anticarcinogenic, antimutagenic compound [53]. Research shows that ellagic acid, which is an anti-carcinogenic, inhibits the growth of cancer cells; it also causes apoptosis or normal cell death in those cancer cells [54].

Structure activity relationship

Ioune et al. (1995) elucidated that the carboxyl group of gallic acid is presumably implicated in distinguishing between normal and cancer cells and the three adjacent phenolic hydroxyl groups should be essential to the cytotoxicity [55]; this confirms the strong cytotoxic activity of gallic acid.

Radical-scavenging activity of phenolics depends, among other factors, on the number and position of hydroxyl (OH) group substitution in the molecules as which called on the antioxidant activity of the flavonoids is known to be associated with the number of free hydroxyg groups (OH); moreover the presence of the carboxyl, alkyl or other groups affects the antioxidant activity of phenol compounds which describes the obtained antioxidant activity results for compounds 1, 4, 5 and 6. The maximum effectiveness for radical scavenging apparently requires the two hydroxyl groups in the ortho-diphenolic arrangement in the B ring and 3-OH group attached to the 2,3-double bond and adjacent to the 4-carboxyl in the C ring. The glycosylation of flavonoids as in case of kaempferol-3-O-α-L-rhamnoside reduces their activity when compared to the corresponding aglycones [56]. From our findings, it seems that free OH group in positions 5, 7, and 4‘ especially position 7, may potentiate the activity against the radical with respect to the presence of glucoside groups, their types, numbers, and positions. While free OH group in positions 5 and 4’ may be associated with the inhibitory activity against radicals. The blocking of position 3 or the steric hindrance of OH group by glucoside groups may lead to dramatic decrease in the activities as shown in compound 7.

Thus, the T AC of methanol extract (polar extract) and isolated pure compounds of A. hirtum leaves showed that this plant can be one of the potential sources of safer natural antioxidants while stronger restrictions are encountered on their application. Hence, replacement of synthetic antioxidants with secondary metabolite phytochemicals exhibiting authoritative and effective antioxidant activities (because of their aspects on human health) from abundantly available plant sources such as A. hirtum may be advantageous.

CONCLUSION

As a conclusion, 10 compounds, methyl gallate (1), cunetaside E (2), bergapten (3), gallic acid (4), ellagic acid (5), EGC (6), kaempferol 3-O-α-L-rhamnoside (7), benzyl-1-0-β-D-glucopyranoside (8), salidroside (9), and β-sitosterol (10) have been successfully isolated from A. hirtum leaves. Our results revealed that compounds 4, 5 and 6 showed significant cytotoxic activity against liver carcinoma; this causes these compounds useful as chemotherapeutics agents not only for their cytotoxic activity but also for their antioxidant potential; these compounds may act in a synergistic manner. Furthermore, ellagic acid is the most antimicrobial active agent among the isolated compounds against four different microbial strains. However, further studies must be necessary to elucidate the mechanism situated with these activities. This report may serve as a footprint concerning the biological and pharmacological activities of A. hirtum leaves.

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