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Bioactive Compounds From Leaves and Bolls Extracts Of *Gossypium Barbadense L.* And Assessment of Their Antioxidants & Cytotoxic Activities

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Abstract

Gossypium barbadense L. has always been a major economic Egyptian crop known as cotton or white gold. The adverse folk medicinal uses and rich phytochemical content previously reported for the plant made it interesting to proceed in the following comparative study between the methanolic leaves and bolls extracts to reveal their polyphenolic content and compare their antioxidant and cytotoxic activities against different cancer cell lines.

The aim of this investigation is to identify the bioactive components in the methanol extracts of leaves and bolls, by using Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-Mass) of methanol extract from bolls , demonstrates the presence of bioactive phenolic compounds namely ; cinnamaldehyde, cinnamyl alcohol, 6-methoxy-7 -hydroxycoumarin, rutin, (+)-gallocatechin , isoquercetin, kaempferol-3-sulfate, epigallocatechin gallate, hydroxybenzoic acid and isorhamnetin-3-*O*-glucuronide and from leaves extract indicate the presence of major compounds, kaempferol, quercetin-3-*O*-pentoside, kaempferol-3-sulfate, quercetin-3-*O*-β-glucoside , quercetin-3-*O*-(6-acetyl-β-glucoside), kaempferol-3-*O*-glucuronide, epigallocatechin gallate, aspalathin, kaempferol-3,7,4'-trimethyl ether, isorhamnetin-3-*O*-β-glucuronide and protocatechuic acid-3-*O*-glucoside.

Additionally, the methanolic extracts of leaves and bolls, were assessed for their *in vitro* anticancer effects on various human cell lines, including colon (HCT116), liver (HEPG2) and breast (MCF-7) cancer cell lines and doxorubicin as the traditional anticancer drug was compared. The outcomes showed that the methanol extract of leaves was more potent on liver (HEPG2) and breast (MCF7) cell lines than bolls extract. Regarding colon (HCT116) cell line the leaves extract has less activity than bolls extract.

The spectrophotometric techniques were used to examine the antioxidant potentials of *G. barbadense* extract, including their scavenging capacity and reduction potential.

Results showed a strong antiproliferation/cytotoxicity index against cancer cell lines when extracted in methanol for both the bolls and leaves extracts.

Keywords: *G. barbadense*, leaves, bolls, LC-ESI-MS, antiproliferative activity

Introduction

The genus *Gossypium*, which belongs to the family Malvaceae and tribe Gossypieae, has around 50 species, four of which are farmed for their spinnable fiber. The remaining 46 species are found in their natural habitats across the world's tropics and subtropics. The wild species of Gossypium are key sources of beneficial features such as specific and superior fibre qualities, cytoplasmic male sterility, tolerance to biotic and abiotic stressors, which may be introgressed into the cultivated species for improvement [1]. The world's four domesticated cottons (Old World diploids such as *G. arboreum* and *G. herbaceum* and New World tetraploids such as *G. barbadense and G. hirsutum)* have been grown independently in different parts of the world [2]. This genus first appeared around 5-10 million years ago [3]. *G. barbadense* L.(Cotton) is a significant economic crop commonly known as white gold. Cotton has a wide variety of applications, principally medical, but also many other uses, such as pigments, animal feed derivatives, different oil extract applications, and so on [4].

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Chemical ingredients in Malvaceae family include *G.barbadense* plant have an active constituent, a sesquiterpene pigment termed gossypol (a dimeric naphthalene derivative), which induces male sterility. Flavonoids, betaine, choline, and salicylic acid are other components of various portions of the plant [5].
The overall *G. barbadense* plant contains important substances such as terpenes, phenolics, fatty acids, lipids, carbohydrates, and proteins [6] .These substances, present in the plant's seeds, bolls, calyx, leaves, stalks, stems, and roots, have significant biological roles in humans and animals[7]. It includes compounds that may be beneficial to people and animals, such as gossypol, a polyphenol with potential contraceptive properties [8] and trans-caryophyllene, a terpenoid with anti-inflammatory and cytotoxic properties [9,10].
By-products created by the *G. barbadense* business may represent a possible source of valuable extractives as a result of the dispersion of chemical compounds throughout the plant. The detected compounds are supplying an increasingly important reservoir of bioactive molecules with antioxidant and antiproliferative potential. *G.barbadense* is used in folk medicine to treat ulcers, malaria, high blood pressure, delayed or irregular periods, convulsions, diarrhoea, and hypertension [11,12].
The current study will concentrate on evaluating the in vitro anticancer and antioxidant activities of *G. barbadense* leaves and bolls extracts in light of the plant's numerous therapeutic applications. Several cell culture and animal model studies reveal that numerous natural substances with various chemical structures can operate as chemo preventive and chemotherapeutic agents. These biologically active chemicals are derived from structural classes such as polyphenols, alkaloids, terpenoids, and organosulfur compounds [13].

As a result, it is expected that traditional medicines will not only continue to contribute new compounds for drug development, but they may also serve as the foundation for a wider adoption of crude extracts in standardized form as another type of medication in modern practice [14].

The in-vitro anticancer activity of methanolic extracts using MTT assay of bolls and leaves of *G. barbadense*, against human colon (HCT116), liver cancer (HEPG2), and breast (MCF7) cell lines as part of ongoing efforts to fully utilize the therapeutic properties of natural products.

Materials and Methods:

General.

All chemicals used are analytical grade and purchased from Sigma-Aldrich, the solvents used were purchased from Merck (Germany).

Plant Collection.

The fresh *G. barbadense* bolls (500 gm) and leaves (1.5 kg) were collected in August 2021 from a natural field in Zagazig city located in Egypt with (30.5877,31.502) coordinates, 63 km in Lower Egypt. Situated in the eastern part of the Nile delta.
The plant identified by Professor Alaaeldin Sayed Ewase, Ministry of Environment. A voucher herbarium specimen (No. M145) was deposited in herbarium of National Research Centre, Giza, Egypt with global code (CAIRC).

Extraction

The collected bolls and leaves were washed to remove impurities such as dust and then dried at 40◦C for 48hr .The dried portions were ground into a fine powder in order to increase the contact area between extracting solvent and the solid material.
The leaves and bolls powder were defatted with petroleum ether (60-80^{o}) then extracted with methanol solvent using Soxhlet equipment. The methanol extracts were concentrated and dried to the final crude methanol extracts of bolls (16.37 g) and leaves (17.48g) were stored at -4°C for future investigations.

Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-Mass)

The study was carried out at the Drug Discovery and Development Research Centre (Ain Shams University, Giza, Egypt), which employed an inverse stage C_{18} column (ACQUITY UPLCBEH C_{18} 1.7 µm particle size 2.1omb 5 mm Column) to separate phenolics and flavonoids from methanolic extract. The sample solution (100 µg/mL) was produced using MeOH grade solvent, ,
The sample (10 µl) was injected into the LC-ESI-MS instrument. Before injecting the sonication, the portable sample stage was filtered and degassed using a 0.2 µm filter membrane disc.
Gradient mobile phase elution was performed using two eluents: H_2O acidified with 0.05% formic acid and acetonitrile at a flow rate of 0.5mL/min. The following parameters were employed in negative ion mode: source temperature 150°C, cone voltage 30 eV, capillary voltage 3 kV, desolation temperature 440°C, cone gas flow 50 L/h, and desolation gas flow 900L/h. The ESI negative ion mode was used to identify mass spectra between m/z 100 and 1000. Peaks and spectra were analysed and tentatively identified using the Maslynx 4.1 software by

comparing retention time (R_t) with mass range and international data.

Assessment of biological activities

Cytotoxicity assay

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [15]. This method was to determine the cytotoxic effect of compounds on 3 human cancer cell lines namely hepatocellular carcinoma cell line) HepG2), Caucasian breast adenocarcinoma (MCF7) and Human Colon Carcinoma (HCT116). The cells were seeded into 96-well culture plates at 10x103 cells/well for cancer cell lines in fresh complete growth medium. Media was aspirated and fresh medium (without serum) was added to the cells. Compounds at different concentrations or Doxorubicin (positive control) were added and the cells incubated for a further 48 h after which the medium was replaced with 40ul MTT salt (2.5µg/ml) were added to each well and incubated for further four hours. To stop the reaction and dissolving the formed crystals, 200µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC. The absorbance was measured at 540 nm on a multiwall scanning spectrophotometer (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA). All incubation steps were carried out in a 37°C humidified incubator with 5% CO2. A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

Hydroxyl Radicals

A slightly modified version of the procedure described in [16] was used to test *G. barbadense* antioxidant activity in reducing hydroxyl radicals. Fenton's reaction between $FeSO_4$ and H_2O_2 produces hydroxyl radicals as a by-product. By setting up a reaction fusion with 1 mL of ferri (II) sulphate 1.5 mM, 0.5 mL of hydrogen peroxide 6 mM, and 20 mM of sodium salicylate, the sample carried out the experiment. Additionally, quercetin as a positive control (10-100 g/mL) and each concentration of the sample solution (10-1000 g/mL) were added. For 30 minutes, the combination maintained the fusion at 37°C. After the incubation period, UV-Visible spectrophotometry at 520 nm was used to measure the absorbance. Determination of antioxidant action by the following equation:

Hydroxyl Radical Inhibition (%) = $[1-(A_1-A_2)/A_0] \times 100$

Where as : A_0 shows the absorbance of the reagent only, A_1 shows the absorbance of the *G. barbadense*, A_2 shows the absorbance in the absence of reagents (sodium salicylate).

***β*–Carotene Degradation**

Using the *β*-Carotene Degradation (BCD) assay, *G. barbadense* extracts were found to have antioxidant action in decreasing lipid peroxidation radicals. 5 mg of *β*-Carotene pollen are included in the *β*-Carotene emulsion, which is then dissolved in chloroform. The mixture was properly upset into 250 mL of distilled water and allowed to swing until a transparent *β*-Carotene emulsion was forming. The ingredients were four mL of polysorbate 20 as a surfactant and 0.5 mL of linoleic acid as a peroxide radical originator (chloroform in the mixture was evaporating). The flask contained a series of ethyl acetate fractions (10–1000 g/mL), and 2 ml of the *β*-Carotene emulsion was added. Additionally, distilled water was added to 5 mL of the combinations in a volumetric flask before being incubated in a 50ºC oven for 20 minutes. After the incubation period, a spectrophotometer is used to measure the absorbance (461 nm). The mixes underwent measurements every 30 min. over a period of 0 to 120 min.. Based on the difference between the decrease rates of the sample and control (*β*-Carotene emulsion only), the antioxidant impact was calculated. Based on the following formula, the samples computed the percentage (%) of blockage of *β*-Carotene reduction rate [17].

Inhibition of Degradation Rate (%) =100 $[1-(A_0-A_t) / (A_{00}-A_{0t})]$

Where : A_0 and A_{00} are the absorbance value measured at zero time of the incubation for test sample and control, respectively, A_t and A_{0t} are the absorbance value measured after incubation for test sample and control, respectively. The results were expressed in percentage of blockage of *β*-Carotene reduction rate.

Analytical Statistics

Version 23 of the Statistical Package for the Social Sciences (SPSS) was used to statistically analyse the data. (Copyrighted by USA-based IBM SPSS program) The information was shown as a mean standard error of mean (SEM).

Results and Discussion

Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-Mass)

The methanolic extract of *G. barbadense* bolls indicate the presence of main components; cinnam-aldehyde, cinnamyl alcohol, 6-methoxy-7–hydroxy-coumarin, rutin, (+)-gallocatechin, isoquercetin, kaempferol-3-sulfate, epigallocatechin gallate,

hydroxybenzoic acid, isorhamnetin-3-*O*-glucuronide and fukugetin, that might have contributed to their therapeutic potential, (Fig.1 , Table1).

On other hand, the methanolic extract of leaves indicate the presence of major compounds, kaempferol, quercetin-3-*O*-pentoside, kaempferol-3-sulfate, quercetin-3-*O*-*β*-glucoside, quercetin-3-*O*-(6-acetyl-*β*-glucoside), kaempferol-3-*O*-glucuronide, epigallocatechin gallate,aspalathin,kaempferol-3,7,4'-trimethyl ether,isorhamnetin-3-*O*-*β*-glucuron ide and protocatechuic acid-3-*O*-glucoside with comparison in reference [18].

The high components of isolated phenolic and flavonoid compounds with varied hydroxyl groups in the flavonoid structure, when paired with a highly conjugated electron system, allow them to operate as free radical scavengers via hydrogen atom or electron donation activities. Furthermore, they can suppress the formation of ROS (reactive oxygen species) such as hydroxyl radicals by chelating redox-active transition metal ions [19]. This has the potential to enhance reduce cancer detection by reducing events such as DNA oxidative damage.

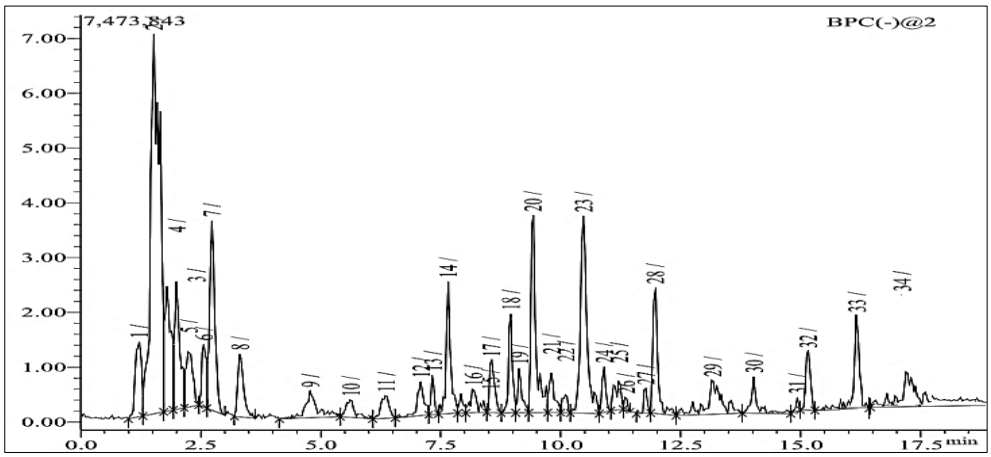

Fig.1. LC-ESI-Mass profile of *G. barbadense L.* bolls methanol extract.

Table 1. Major Bioactive Phenolic Compounds Of *G. Barbadense* Bolls Extract Identified By LC-ESI-Mass

Peak No.	$R_{t\ min}$	Conc. (%)	Base Peak m/z	Exact Mass (g/mol)	Structure formula	Identification Compounds
1.	1.52	8.72	131.10	132.05751	C_9H_8O	Cinnamaldehyde
2.	1.79	1.95	133.05	134.17799	$C_9H_{10}O$	Cinnamyl alcohol
3.	2.73	2.53	191.05	192.04227	$C_{10}H_8O_4$	6-Methoxy-7 -hydroxycoumarin
4.	4.78	0.78	609.20	610.15338	$C_{27}H_{30}O_{16}$	Rutin
5.	7.65	1.47	305.15	306.07394	$C_{15}H_{14}O_7$	(+)-Gallocatechin
6.	9.42	2.56	463.15	464.37900	$C_{21}H_{20}O_{12}$	Isoquercetin
7.	10.47	3.22	365.10	366.17053	$C_{15}H_{10}O_9S$	Kaempferol -3-Sulfate
8.	11.97	1.55	457.25	458.08490	$C_{22}H_{18}O_{11}$	Epigallocatechin gallate
9.	15.16	2.23	137.05	138.03169	$C_7H_6O_3$	Hydroxybenzoic acid
10.	16.17	3.22	491.20	492.38901	$C_{22}H_{20}O_{13}$	Isorhamnetin-3-*O*-glucuronide

Assessment of biological activities

In-*vitro* cytotoxic activities

In comparison to standard anticancer drug (doxorubicin) , the in *vitro* cytotoxic effect of bolls and leaves methanol extracts were evaluated for their antiproliferative potential towards different human cell lines, including colon (HCT116), liver (HEPG2), and breast (MCF-7) cancer cell lines. Results in (Table 2) showed that the leaves methanol extract had more antiproliferative action against liver (HEPG2) and breast (MCF-7), while lower against colon (HCT116) than extract from bolls. The methanolic extract from bolls demonstrated a potent anti-multiplication cytotoxicity effect on three cancer cell lines **(**Fig. 2**)**, as evidenced by the low IC_{50} values (22.8 ± 7.04, 30.1 ± 4.05 and 67.5 ± 7.1 µg/mL, respectively), whilst the IC_{50} effect of methanolic extract from leaves is demonstrated by values (19.4 ± 3.7, 25.6 ± 4.3 and 99.41 ± 1.87µg/mL, respectively in comparison to doxorubicin drug is demonstrated by values (2.7 ± 0.06 , 5.03 ± 0.7and 4.8 ± 0.6) against human cancer cell lines (HEPG2, MCF-7 and HCT116), respectively.

Table 2. In *vitro* cytotoxic activity of leaves and bolls of *G. barbadense* towards human colon, liver and Breast cell lines.

Extract	Colon (HCT116) IC_{50} [µg/ml]	Liver (HEPG2) IC_{50} [µg/ml]	Breast (MCF7) IC_{50} [µg/ml]
Bolls	67.5 ± 7.1	22.8 ± 7.04	30.1 ± 4.05
Leaves	99.41 ± 1.87	19.4 ± 3.7	25.6 ± 4.3
Doxorubicin	4.8 ± 0.6	2.7 ± 0.06	5.03 ± 0.7
DMSO	N.A.	N.A.	N.A.

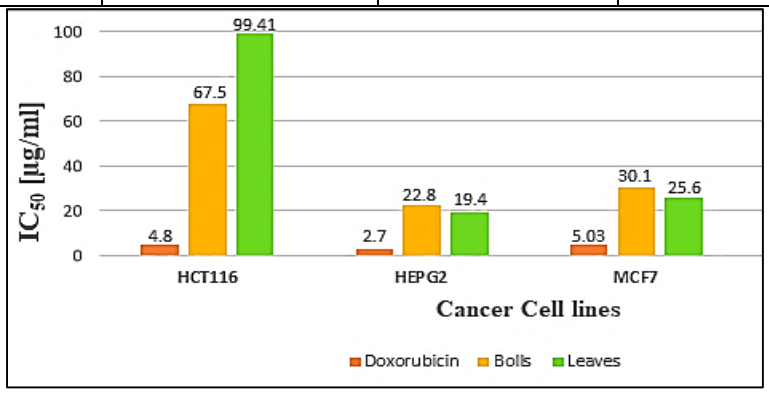

Fig. 2. IC_{50} of methanol extracts of *G. barbadense*, leaves and bolls in comparison to Doxorubicin on the HCT116, HEPG2 and MCF-7 cancer cells

At the clinical level, current anticancer medicines are incredibly cytotoxic and have major deleterious effects on many human organs. As a result of these constraints on the use of synthetic anticancer chemicals, new medicinal plant extracts must be screened and developed as alternatives that are less harmful to normal cells, have a higher therapeutic index, a different mechanism of action, and shorter treatment cycles.

The present study's conclusions state that the crude extract of *G. barbadense* clearly demonstrated a cytotoxic impact on cancer cell lines of various sorts, with greater potency than normal cell lines. This medication has anticancer characteristics, especially against melanoma and bladder carcinoma, and has been used as a male oral contraceptive [20,21].

Gossypol is highly cytotoxic in several tumor cell lines, according to several research [22]. Uncertainty surrounds the biochemical and molecular mechanisms underlying such cytotoxic/ antiproliferative biological effects. Given the significance of these pathways in cancer cell death, significant efforts are being undertaken to apply this knowledge to the rational design and development of novel treatment approaches to improve the efficacy of chemotherapeutic drugs [23].

Antioxidant potentials

The high concentrations of phenolics and flavonoids increase the plant's capacity of their antioxidant properties [24].

Our previous study reported that the methanolic extract of *G. barbadense* leaves have has a maximum higher phenolic acids content as compared to bolls extract [25].

Checking for Hydroxyl Radicals

The Fenton reaction mechanism, which involves the reaction between Fe^{2+} and H_2O_2 to form hydroxyl radicals, serves as the basis for reducing hydroxyl radicals. By using a spectrophotometer, the 2,3-dihydroxy-benzoic acid and 2,5-dihydroxy-benzoic acid that result from the reaction between the hydroxyl radicals and salicylates were quantified.

The findings of the research on lowering hydroxyl radicals demonstrate that the strength of methanolic extract from *G. barbadense* leaves (Fig. 3). The greatest concentration, 1000 g/ml, has significant effects with 79.3 % percent inhibition value.

The IC_{50} value of quercetin as a contrast was 2.32 g/ml (50 g/ml), extreme antioxidant levels, and inhibiting hydroxyl radicals up to 50% (IC_{50}) required a concentration of 121.4 g/ml (100-150 g/ml), a moderate degree of antioxidant strength.

β- Carotene Degradation Assay

Linoleic acid produces hydroperoxides, which are used in the beta-carotene technique of testing for antioxidant activity to produce free radicals. Free radicals are produced during linoleic acid oxidation as a result of the hydrogen atom being removed from one daily methylene group. This causes β-Carotene to oxidise, which results in the loss of the chromophore group, which gives orange foods their colour.

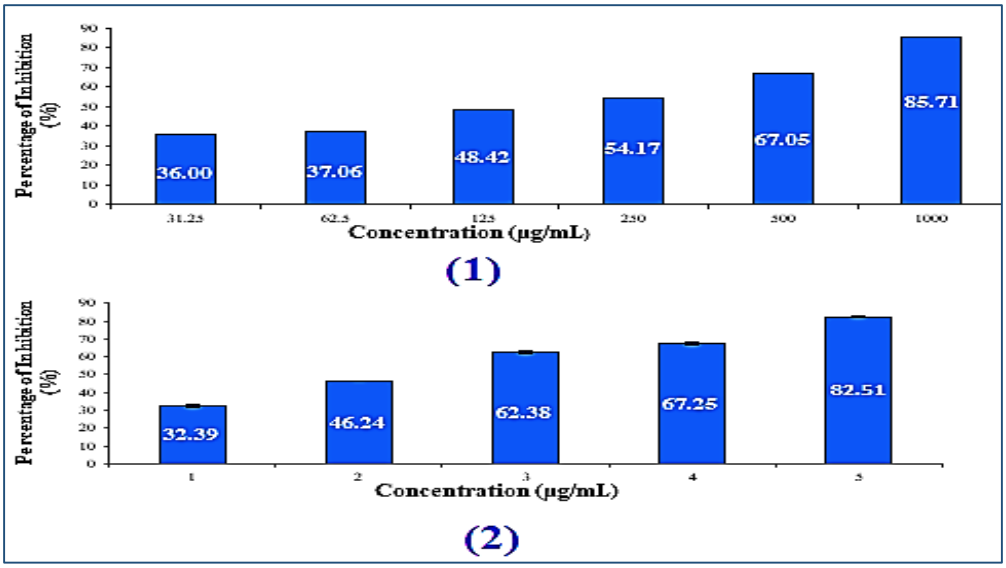

Fig. 3. Graph of the relationship between sample concentration and % of inhibition of G. *barbadense* leaves (1) and standard quercetin (2). The data with triplicate (n=3).

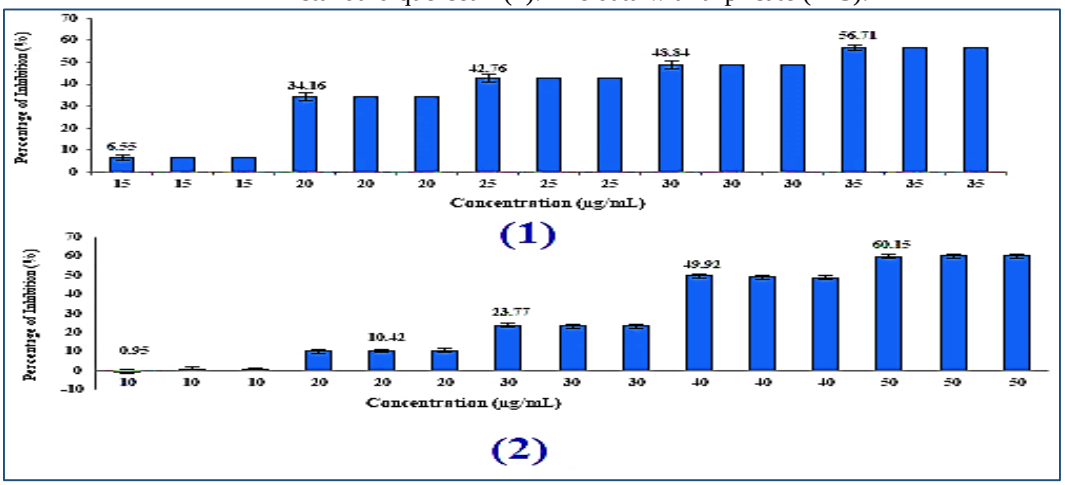

Fig. 4. Graph of inhibition percentage and sample concentration of *G. barbadense* leaves (1) and positive control of quercetin (2). The data with triplicate (n=3).

Based on the IC_{50} value, antioxidant activity testing using the Binary Coded Decimal, or BCD method can be quantitatively evaluated. The IC_{50} value is used to determine the sample's concentration at which 50% of radicals are inhibited. Antioxidant capability demonstrates that a sample's potential to reduce free radicals increases with a reduced IC_{50} value.

In (Fig.4), the findings of this investigation are displayed. In comparison to quercetin, which has an IC_{50} rate of 43.38 g/mL, *G. barbadense* leaves extract can slow down the colour deterioration of *β*-carotene. According to the findings, the sample's IC_{50} value has approximately the same potential as a quercetin positive control. It is as a result of the samples' high flavonoid and phenolic content.

Conclusion

The spectroscopic analysis, LC-ESI-MS showed that the methanolic extract from bolls and leaves of *G. barbadense* have the highest contents of phenolic, flavonoids, and their glycosides with potent antioxidant activity which can be an excellent choice for biological and chemical analysis and can be further subjected for the isolation of the therapeutically active compounds with anticancer potency.

The in-*vitro* anticancer efficiency of methanol extracts from bolls and leaves were evaluated for their antiproliferative potential towards several human cell lines, including colon (HCT116), liver (HEPG2), and breast (MCF7) cancer cell lines, in

comparison to Doxorubicin, the traditional anticancer *G. barbadense* methanol extract from leaves was reported to have stronger antiproliferative action against liver (HEPG2) and breast (MCF-7), while lower against colon (HCT116) than methanol extract from bolls.

Additionally, scientists have long been affected by conventional plant-based folk remedies as they look for new drugs to maintain and improve human health.

References

[1] Gotmare, V., Singh, P., & Tule, B. Wild and cultivated species of Cotton. Technical Bulletin; Central Institute for Cotton Research: Nagpur, India, (2000).

[2] David S. Senchina, Ines Alvarez, Richard C. Cronn, Bao Liu, Junkang Rong, Richard D. Noyes, Andrew H. Paterson, Rod A. Wing, Thea A. Wilkins, Jonathan F. Wendel Rate Variation Among Nuclear Genes and the Age of Polyploidy in Gossypium . Molecular Biology and Evolution, (2003).20(4),633-643., https://doi.org/10.1093/ molbev/msg065

[3] Wendel, J. F., Brubaker, C., Alvarez, I., Cronn, R., & Stewart, J. M. Evolution and natural history of the cotton genus. In Genetics and genomics of cotton (3-22). Springer, New York, NY. (2009). https://link.springer.com/chapter/ 10.1007/978-0-387-70810-2-1.

[4] Sun, W., Shahrajabian, M. H., Khoshkharam, M., Shen, H., & Cheng, Q. Cultivation of cotton in China and Iran with considering biological activities and its health benefits. Cercetari Agronomice in Moldova, (2020). 1(181), 105-120. DOI: 10.46909/cerce-2020-009.

[5] Das, U., & Islam, M. S. A review study on different plants in Malvaceae family and their medicinal uses. Am. J. Biomed. Sci. Res, (2019).3(2),94-97.DOI:10.34297/AJBSR.2019. 03.000641.

[6] Emmanuel, E. E., Sherifat, O. A., & Isiaka, A. O. Constituents and antimicrobial properties of the leaf essential oil of Gossypium barbadense (Linn.). Journal of Medicinal Plants Research, (2011). 5(5), 702-705. https:// academicjournals. org/journal/JMPR/article-full-text-pdf/CD3C5A 718760.

[7] Suijun, W., Zhen, Y., Ying, G., & Yanfang, W. A role for trans-caryophyllene in the moderation of insulin secretion. Biochemical and Biophysical Research Communications, (2014). 444(4), 451-454. https://doi.org/ 10.1016 /j.bbrc.2013.11.136.

[8] Han, X., Shen,T.,Lou, H. Dietary polyphenols and their biological significance. International journal of molecular sciences, (2007).8(9),950-988. https:// doi. org/10.3390/i8090950.

drug.

[9] Amiel, E., Ofir, R., Dudai, N., Soloway, E., Rabinsky, T.,Rachmilevitch, S. β-Caryophyllene, a compound isolated from the biblical balm of gilead (Commiphora gileadensis), is a selective apoptosis inducer for tumor cell lines. Evidence-based complementary and alternative medicine, (2012). https://doi.org/10.1155/2012/872394.

[10] Elizabeth S. Fernandes , Giselle F. Passos , Rodrigo Medeiros , Fernanda M. da Cunha , Juliano Ferreira , Maria M. Campos , Luiz F. Pianowski d, João B. Calixto. Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of Cordia verbenacea European journal of pharmacology, (2007). 569(3), 228-236. https://doi.org/10.101 6 / j.ejphar.2007.04.059.

[11] Ade-Ademilua, O. E.,Okpoma, M. O. Gossypium hirsutum L. and Gossypium barbadense L.: differences in phytochemical contents, antioxidant and antimicrobial properties. Ife Journal of Science, (2018). 20(1), 77-88. DOI: 10.4314/ijs.v20i1.8.

[12] Salako, O. A., & Awodele, O. Evaluation of the antimalarial activity of the aqueous leaf extract of Gossypium barbadense (Malvaeceae) in mice. Drugs and Therapy Studies, (2012). 2(1), e2-e2. https://doi.org /10. 4081/dts.2012.e2.

[13] Nawwar, M. A.; Hussein, S. A.; El-Mousallami, A. M.; Hashim, A. N.; Mousa, M. A.; Hetta, M. H.; Hamed, M. A.; Werner, V.; Becker, A.; Haertel, B.; Lindequist, U. Phenolics from Caesalpinia ferrea Mart.: antioxidant, cytotoxic and hypolipidemic activity Die Pharmazie , (2015). 70 (8),553–558.doi: 10.1691/ph. 2015. 5526.

[14] Bone, K.,Mills, S. Principles and practice of phytotherapy: modern herbal medicine. Elsevier Health Sciences, (2012).

[15] Mosmann, T. Rapid colorimetric assays for cellular growth and survival: Application proliferation and cytotoxicity assays. J Immunol Methods, (1983).65,55-63.

[16] Talha Ali Chohan , Muhammad Sarfraz, Kanwal Rehman , Tariq Muhammad , Muhammad Usman Ghori , Kashif Maqbool Khan , Iftikhar Afzal , Muhammad Sajid Hamid Akash , Alamgeer i, Arif Malik . Tahir Ali Chohan. Phytochemical profiling, antioxidant and antiproliferation potential of Euphorbia milii var.: Experimental analysis and in-silico validation, Saudi Journal of Biological Sciences,(2020)27(11),3025-3034 https: //doi. org/10.1016/j.sjbs.2020.08.003 .

[17] Rosidah, Yam, M., Sadikun, A., Asmawi, M. Antioxidant potential of Gynura procumbens. Pharmaceutical Biology, (2008). 46(9),616-625. https://doi.org/10.1080/13880200802179 642.

[18] Amani M.D Elmesallamy; Mohamed El-Zaidy; Mohamed M.D Younes ; Sahar A.M Hussein . Green Biosynthesized Selenium Nanoparticles and Bioactive Compounds by Gossypium barbadense L Extract and Their Cytotoxic Potential. Egyptian Journal of Chemistry, (2022).Doi.10.21608/EJCHEM.2022.171646.7132

[19] McPhail D.B., Hartley R.C., Gardner P.T., Duthie G.G. Kinetic and stoichiometric assessment of the antioxidant activity of flavonoids by electron spin resonance spectroscopy. J. Agric. Food Chem., (2003).51: 1684–1690.https://doi.org/10.1021/jf0259 22v

[20] Tripathi, K. D. Essentials of medical pharmacology. JP Medical Ltd, (2013).

[21] Prakash, O. M., Kumar, A.,Kumar, P. Anticancer potential of plants and natural products. Am J Pharmacol Sci, (2013). 1(6), 104-115.DOI:10.126 91 /ajps-1-6-1.

[22] Ping Gao,Chantal Bauvy,Sylvie Souquere, Giovanni Tonelli LeiLiu, Yushan Zhu , Zhenzhen Qiao, Daniela Bakula, Tassula Proikas-Cezanne, Gerard Pierron, Patrice Codogno, Quan Chen and Maryam Mehrpour, The Bcl-2 Homology Domain 3 Mimetic Gossypol Induces Both Beclin 1-dependent and Beclin 1-independent Cytoprotective Autophagy in Cancer Cell. Journal of Biological Chemistry, (2010). 285(33), 25570-25581. DOI:10.1074/jbc. M110.11812

[23] Lee, K. H. Discovery and development of natural product-derived chemothera- peutic agents based on a medicinal chemistry approach. Journal of natural products, (2010). 73(3), 500-516.https://doi.org/10.1021/np900821e.

[24] Sahar A.M.Hussein , Amani M.D Elmesallamy , Abdelmohsen M.Soliman , Mohamed El-Zaidy, Mohamed El Gerby. Bioactive Compounds from Mango Peels (Mangifera Indica Tommy Atkins) and Demonstration Of Its Cytotoxicity And Ccl4 Induced Hepatotoxicity In Rats. Egyptian Journal of Chemistry (2021). 64(9), 4747-4756. DOI: 10.21 608/ EJCHEM. 2021. 73347 . 3632.

Amani M.D.El-Mesallamy , Mohamed I.M. El-Zaidy , Mohamed El-Garby Younesa , Sahar A.M Hussein. Demonstration of Phenolics Content from Different Parts of Gossypium barbadense L. Plant and Evaluation of Their Antimicrobial Activity, Bulletin of Faculty of Science, Zagazig University (BFSZU) (2023), 1(1),169175,doi:10.21608/bfszu.2022.169411.1195