Bulletin of Faculty of Science ,Zagazig University (BFSZU) **2023**
Bulletin of Faculty of Science, Zagazig University (BFSZU)
e-ISSN: 1110-1555
Volume-2023, Issue-1, pp-169-175
Research Paper DOI: **10.21608/bfszu.2022.169411.1195**

Demonstration of Phenolics Content from Different Parts of *Gossypium barbadense L.* Plant and Evaluation of Their Antimicrobial Activity

Amani M.D.El-Mesallamy [a], Mohamed I.M. El-zaidy[a]* , Mohamed El-Garby Younes[a], Sahar A.M Hussein[b]

[a]Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt.
[b]Department of Phytochemistry and Plant Systematics; National Research Centre, Giza, Egypt.
Corresponding author: moh.elzainy@gmail.com

ABSTRACT **: The purpose of this work was to estimate the total phenolics and flavonoids content of alcoholic extracts of *Gossypium barbadense L.* parts such as bolls, flowers and leaves. The presence of several sorts of phenolics, flavonoids, tannins, carbohydrates, glycosides and saponins were shown by qualitative phytochemical screening of the methanolic extracts, whereas alkaloids, terpenoids, and steroids were lacking. The maximum total phenolics content (0.320±0.012 mg GAE/g) was determined in Gossypium barbadense L. leaves extract, whereas the highest total flavonoids content (0.488±0.024mg QE/g) was determined in its bolls extract. Since phenols and flavonoids are potentially active, we assessed the antimicrobial activities of methanolic extracts of *Gossypium barbadense L.* parts such as bolls, flowers and leaves. Methanolic extracts were examined for antibacterial activities against (*Escherichia coli* ATCC10536, *Staphylococcus aureus* ATCC6538, *Salmonella enterica* ATCC 14028, and *pesudomonas aeruginosa* ATCC 9027), whereas antifungal activities against two fungi (*Aspergillus niger, Aspergillus fumigatus*) using the disc diffusion method. The results were compared to the zone of inhibition caused by standard antibiotics that are commercially accessible. The results demonstrated that methanolic extracts of Gossypium barbadense L. parts such as bolls, flowers and leaves exhibit antibacterial activity against E.coli and S.aureus, whereas only the bolls and flowers extracts have action against P.aeruginosa. While the growth of S.enterica was inhibited by bolls extract only. On other hand, there are no anti-fungal properties against *Aspergillus niger* and *Aspergillus fumigatus* for all methanolic extracts of *Gossypium barbadense L.* parts.**

KEYWORDS: phytochemicals; Gossypium barbadense L. parts;total phenolics; total flavonoids;antimicrobial activities

---

Date of Submission: 18-10-2022 Date of acceptance: 27-11-2022

I. INTRODUCTION

Cotton plant, commonly known as *Gossypium* species, is divided into two groups: cultivated and wild. They are members of the Malvaceae family and comprise around fifty (50) species; G. *hirsutum*, G. *herbaceum*, G. *barbadense*, and G. *arboreum* are used for both commercial and therapeutic uses [1]. *Gossypium barbadense* Linn is a 1-3 m in height perennial under shrub native to South America that is currently found from Senegal to Nigeria and is commonly planted in the tropics. The leaf infusion is beneficial as an antidote for colds and bronchitis, and the young shoots are pulped for palpitations, wound dressings, and the treatment of systemic diarrheas [2]. *Gossypium barbadense* leaves are used to treat hypertension and irregular or delayed menstruation [3]. Additionally, *Gossypium barbadense* aqueous leaf extract exhibits a mild suppressive antimalarial action [4]. Cotton is vulnerable to insect, herbivore, and disease invasion. As a result of the plant's resistance to these predators, it develops compounds that are utilised as a defence tactic. Terpenes are an important class of defensive chemicals produced by the cotton plant [5, 6]. Cottonseed is utilised as a raw material in the spinning and weaving industries in the area. It is also used to treat emetic diseases, veneral disorders, malignancies, paralysis, epilepsy, convulsions, spasms and cutaneous and subcutaneous parasite infection [7]. It has antifungal characteristics, and it is occasionally used as a male anti-fertility medication [8, 9]. *Gossypium barbadense* has yielded several bioactive triterpenoids and sesquiterpenoid aldehyde compounds that have been isolated and described [10]. Many of these compounds have demonstrated biological features such as antibacterial, insecticidal, and cytotoxic activity. The chemical composition of *Gossypium barbadense* essential oil from Nigeria indicated the existence

of nineteen components, accounting for 92.6 percent of the total oil fraction. Some of the species studied showed considerable antibacterial activity for the oil [10]. As part of ongoing attempts to fully exploit natural products' therapeutic qualities, The goal of this study was to look for phytochemicals and determine the total phenolics and flavonoids content of methanolic extract of parts *Gossypium barbadense L.*, namely bolls, flowers and leaves. Additionally, the antibacterial characteristics of methanolic extracts were assessed against (*Escherichia coli* ATCC10536, *Staphylococcus aureus* ATCC6538, *Salmonella enterica* ATCC 14028 and *pesudomonas aeruginosa* ATCC 9027), as well as the antifungal activity against two fungi (*Aspergillus niger* and *Aspergillus fumigatus*).

II. MATERIALS AND METHODS

Plant Collection

The fresh parts of *Gossypium barbadense L.*, including bolls, flowers, and leaves were harvested between June and August 2021, from a cultivated field in Zagazig - Sharqia, Egypt which were identified and authenticated by Professor of Plant Taxonomy, Alaaeldin Sayed Ewase, Ministry of Environment. A voucher herbarium specimen (No. M145) was placed in herbarium of National
Research Center, (NRC), Giza, Egypt with global code (CAIRC).

Extraction of *Gossypium barbadense L.* parts

Dried parts of *Gossypium barbadense L.*, namely bolls, flowers, and leaves were washed multiple times with double distilled water and air dried at room temperature. Aerial components were carefully crushed into a powder. Each powdered sample (100 g) was defatted in 60-80º petroleum ether, followed by n-hexane. Using the Soxhlet apparatus, the residue was extracted with methanol. The extraction procedures were continued until the solvent in the Soxhlet apparatus's syphon tube became colorless. The crude extracts were filtered and concentrated till dryness using Rotavapor® (Heizbad Hei-VAP, Heidolph, Germany). The extracts were put in vials and kept at -4°C for later investigation.

Chemicals Reagent

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

I. Chemical Studies

1. Phytochemical analysis

Methanolic extracts of parts *Gossypium barbadense L.* such as bolls, flowers and leaves were exposed to phytochemical screening for the presence of phenolics, flavonoids, alkaloids, tannins, saponins and secondary metabolites According to the procedures outlined by [11,12] (Table 1).

2. Quantitative analysis

Quantitative analysis is a useful approach for determining the amount of phytoconstituents contained in plant extracts. It was carried out in Chemistry Department, Faculty of Science, Zagazig University, Egypt.

2.1-Total phenolics content estimation

A modified Folin-Ciocalteu method was used to assess the extracts' total phenolic content (TPC) [13]. 50 mL of methanol was used to dilute 50 mg of gallic acid and aliquots containing 25- 125µg/ml were the result. 100 ml of methanol were used to dilute 1 gm of dry extract before filtering and increasing the volume to 100 ml. 1 ml (1 mg/ml) of this extract was used to determine the phenol levels. 1 ml of the extract or standard was combined with 4 ml of sodium carbonate (75g/l) and 5 ml of the Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v). The mixture was vortexed for 15 seconds, and then the colour was allowed to develop for 30 minutes by letting it sit at 40°C. A UV spectrophotometer was used to detect the absorbance at 765 nm.

2.2-Total flavonoids content estimation

The aluminum chloride technique was used to ascertain the total flavonoids content (TFC) of the extracts [13]. In 50 ml of methanol, 50 mg of quercetin was dissolved and various aliquots containing 25–125 g/ml of methanol were created. 100 ml of methanol were used to dilute 1 g of dry extract before filtering and increasing the volume

to 100 ml. The flavonoids in 1 ml (1 mg/ml) of this extract were calculated. After mixing 3 ml of extract or standard with 1 ml of a 2% $AlCl_3$ methanolic solution, the absorbance at 420 nm was measured using a UV spectrophotometer.

II. Biological studies

1. Evaluation of Antimicrobial Activity

Antimicrobial activity was carried out in Botany Department, Faculty of Science, Zagazig University, Egypt. The antimicrobial activity of methanolic extracts parts such as bolls, flowers and leaves of *Gossypium barbadense L.* was determined by the disc diffusion method [15]. Tested bacterial strains were (*Escherichia coli* ATCC10536, *Staphylococcus aureus* ATCC6538, *Salmonella enterica ATCC* 14028 and *pesudomonas aeruginosa* ATCC 9027), while the anti-fungal activities of different extracts were tested against two fungi (*Aspergillus niger* and *Aspergillus fumigatus*). Each of the three extracts was dissolved in DMSO and a solution of 1 mg/ml concentration was prepared individually. Paper discs of Whatman filter paper were cut to standard size (5cm) and sterilized in an autoclave. Pure colonies were injected with a sterile loop into 5 ml of sterile LB (Luria- Bertani) and cultured for 10-18 hours at 37°C. Microorganisms were cultivated overnight in LB and adjusted to an optical density of 0.5 McFarland standards. 0.1ml of microorganism's suspension was dispersed on LB agar plates using sterile swaps (Sigma-Aldrich) and 0.1 ml of extract is impregnated onto paper discs 6 mm in diameter, following that, the impregnated discs were put on the agar plates using sterile forceps to space the discs 2 cm apart. Plates were refrigerated for 2 hours to allow the tested chemical to diffuse into the agar. The plates were then incubated at 37°C for 16-18 hours. After incubation, zones diameters of inhibition zone were measured and recorded (in mm). The bactericidal activity of a typical standard antibiotic was measured using the same approach and solvents as described previously. The complex's % activity index was determined using the formula shown below:

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

Statistical Analysis

SPSS version 23 of the Statistical Package for the Social Sciences was used to statistically analyse the data (copyrighted by IBM SPSS software, USA). The information was displayed as a mean and standard error of the mean (SEM).

III. RESULTS AND DISCUSSION

I. Chemical Studies

1-Preliminary Phytochemical Screening:

Phytochemical screening of methanolic extract of parts of *Gossypium barbadense L.*, namely bolls, flowers and leaves revealed the presence of several phytoconstituents such as phenolic acids, flavonoids, tannins, glycosides and/or carbohydrates and saponins compounds as indicated in table (1).

Table 1: Results of preliminary phytochemical screening of methanolic extract from different Parts of *Gossypium barbadense L.* Plant

Phytochemical	Tests	Methanolic extract of *Gossypium barbadense L.*		
		Bolls	Flowers	Leaves
Phenolic acids	Ferric Chloride test	++	+++	+++
Flavonoids	Shinoda test	+++	+++	+++
	Alkaline reagent test	+++	++	+++
Tannins	Ferric Chloride test	++	+++	++
Alkaloids	Dragendroff's test	-	-	-
	Hager's test	-	-	-
Carbohydrates	Molisch's test	+++	+++	+
	Fehling test	++	++	+
	Benedict's Test	++	++	+
Glycosides	Keller- Kilani test	++	+++	+
Terpenoids and Steroids	Liebermann-Burchard's test	-	-	-
Saponins	Froth test	+	+	+

(+++)= Present in high concentration, (++) = Moderately Present, (+) = Trace, (-) = Absent

Plant extracts were found to include constituents known to have physiological and therapeutic effects after being subjected to phytochemical analysis [15]. The plant extracts contained phytochemicals, including phenolic acids, flavonoids, tannins, glycosides and/or sugars and saponin compounds. One of the most numerous and common types of plant metabolites is phenolic chemicals. In addition to their antioxidant or free radical terminator activity, flavonoids and other plant phenolics have been shown in the literature to have a number of other biological effects [16]. Tannins interact to proline-rich proteins and stop the synthesis of such proteins. Plants produce flavonoids, which are hydroxylated phenolic compounds, in response to microbial infection. Flavonoids have been demonstrated in vitro to be antibacterial against a variety of diseases. Their ability to interact with soluble and extracellular proteins as well as the bacterial cell wall is most likely what causes them to behave [17]. Additionally, it was discovered that the plant extracts included saponins, which are known to have anti-inflammatory effects [18]. Glycosides lower blood pressure, according to numerous research [19].

The findings of this study show that the identified phytochemical substances may be the bioactive ingredients, and these plants are proving to be an increasingly useful reservoir of bioactive chemicals with significant medical value.

2-Estimation of total phenolic and flavonoids content

2.1-Total Phenolic content estimation

The total phenolic acid contents of the methanol extracts of parts *Gossypium barbadense L.*, namely bolls, flowers and leaves in terms of gallic acid equivalent were 0.244±0.016, 0.296±0.022 and 0.320±0.012 mg GAE /g of extract powder, respectively (Table 2). *Gossypium barbadense L.* leaves showed the maximum phenolic acids content as compared to *Gossypium barbadense L.* flowers and bolls.

2.2-Total flavonoids content estimation

The total flavonoids contents of the methanol extracts of parts *Gossypium barbadense L.*, namely bolls, flowers, and leaves in terms of quercetin equivalent were 0.488±0.024, 0.380±0.014 and 0.430±0.018 mg QE/g of extract powder, respectively (Table 2). *Gossypium barbadense L.* bolls showed the maximum flavonoids content as compared to *Gossypium barbadense L.* flowers and leaves.

Table 2: Total phenolic and flavonoids content of methanolic extract from different Parts of *Gossypium barbadense L.* Plant

Estimation	Methanolic extract *Gossypium barbadense L.*		
	Bolls	**Flowers**	**Leaves**
Total Phenolics (mg GAE/g)	0.244±0.016	0.296±0.022	0.320±0.012
Total Flavanoids (mg QE/g)	0.488±0.024	0.380±0.014	0.430±0.018

Each value represents the average of three analyses ± standard deviation.

The high concentrations of phenolic acids and flavonoids increase the plant's capacity to protect itself against insects, fungus, bacteria and viruses because of their antioxidant properties [20].

II. Biological studies:

1. Evaluation Antimicrobial Activities

The antimicrobial activities of methanolic extracts from various parts of *Gossypium barbadense L.* were tested against several human pathogenic microorganisms using the disc diffusion agar method and the diameter of the inhibition zones was measured and documented in table (3) and Fig. (1). All results were compared to the zone of inhibition generated by commercially available conventional antibiotics.

Table 3: Antimicrobial activities of methanolic extracts from different Parts of *Gossypium barbadense L.* Plant

Extracts	*S.aureus*		*E.coli*		*P.aeruginosa*		*S.enterica*		*A. niger*		*A. fumigatus*	
	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index
Bolls extract	15	75	30	100	10	35.71	5	29.41	NA	----	NA	----
Flower extract	20	100	25	83.33	5	17.85	NA	----	NA	----	NA	----
Leaves extract	10	50	15	50	NA	----	NA	----	NA	----	NA	----
Ciprofloxacin	20	100	30	100	28	100	17	100	NA	----	NA	----

NA → No Activity.

All extracts showed antibacterial activity against *S.aureus* and *E.coli*, while only bolls and flowers extracts displayed activity against *P.aeruginosa*. Furthermore, only bolls extract reduced the development of *S.enterica*. With regards to *S.aureus*, flowers extract displayed the highest antibacterial activity (20 mm), followed by bolls extract (15 mm), and leave extract (10 mm). While bolls extract showed high antimicrobial activity against *E.coli* (30 mm), followed by flower extract (25 mm), and leaves extract(15 mm). Only bolls and flowers extracts displayed antibacterial activity against *P.aeruginosa* (10 and 5 mm), respectively. Furthermore, bolls extract shown efficacy against *S.enterica* (5mm). On other hand, there are no anti-fungal activities of all methanolic extracts of *Gossypium barbadense L.* parts. against *Aspergillus niger* and *Aspergillus fumigatus*.

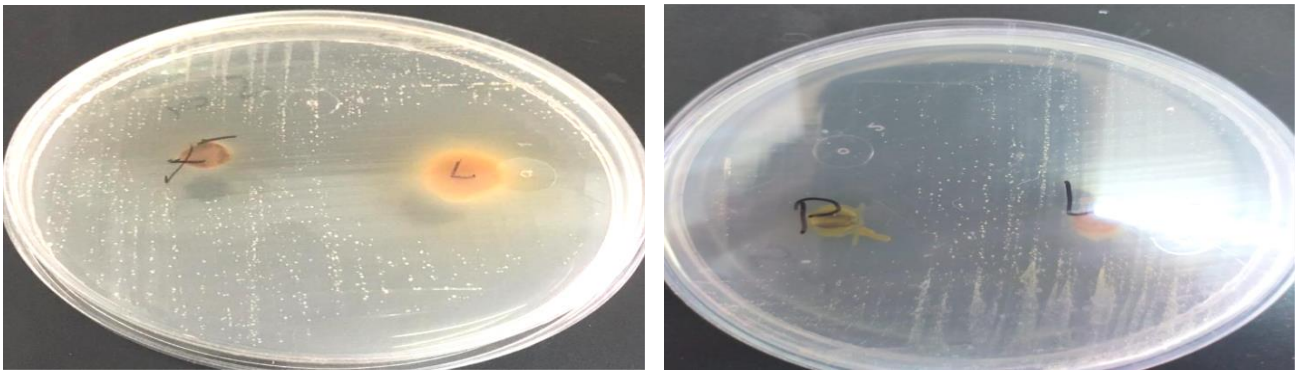

Fig (1): Illustration of the antimicrobial activity of some effective extracts against various human pathogenic microorganisms using agar well diffusion method.

V –CONCLUION

The results of this study suggest that phenolic acids, flavonoids, tannins, glycosides and/or carbohydrates, and saponins are present in the methanolic extract of certain plant sections of *Gossypium barbadense L*., but alkaloids, terpenoids and steroids are not. Furthermore, The methanolic bolls extract shows antibacterial activity against all the test bacterial strain, while flowers extracts displayed activity against all bacterial strain except *S.enterica* . In addition, leaves extracts showed antibacterial activity against *S.aureus* and *E.coli* only . Antibacterial activity may be due to presence of high flavonoids content compared to phenolic content. In contrast, all methanolic extracts of *Gossypium barbadense L.* parts have no antifungal activity against *Aspergillus niger* and *Aspergillus fumigatus*.

REFERENCES

[1] **Gotmare, V., Singh, P., & Tule, B. (2000).** Wild and cultivated species of Cotton. Technical Bulletin; Central Institute for Cotton Research: Nagpur, India, 5.

[2] **Busari, A. A. (1984).** An annotated booklet of some medicinal plants of South-west Nigeria.

[3] **Hasrat, J. A., Pieters, L., & Vlietinck, A. J. (2004).** Medicinal plants in Suriname: hypotensive effect of *Gossypium barbadense*. Journal of pharmacy and Pharmacology, 56(3), 381-387.

[4] **Salako, O. A., & Awodele, O. (2012).** Evaluation of the antimalarial activity of the aqueous leaf extract of *Gossypium barbadense* (Malvaeceae) in mice. Drugs and Therapy Studies, 2(1), e2-e2.

[5] **Opitz, S., Kunert, G., & Gershenzon, J. (2008).** Increased terpenoid accumulation in cotton (*Gossypium hirsutum*) foliage is a general wound response. Journal of Chemical Ecology, 34(4), 508-522.

[6] **Degenhardt, J., Köllner, T. G., & Gershenzon, J. (2009).** Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. Phytochemistry, 70(15-16), 1621-1637.

[7] **Burkill, H. M. (1985).** The useful plants of West Africa. Royal Botanical Gardens, Kew, 1, p319.

[8] **Stipanovic, R. D., Lopez, J. D., Dowd, M. K., Puckhaber, L. S., & Duke, S. E. (2006).** Effect of racemic and (+)-and (−)-gossypol on the survival and development of Helicoverpa zea larvae. Journal of Chemical Ecology, 32(5), 959-968.

[9] **Coyle, T., Levante, S., Shetler, M., & Winfield, J. (1994).** In vitro andin vivo cytotoxicity of gossypol against central nervous system tumor cell lines. Journal of neuro-oncology, 19(1), 25-35.

[10] **Emmanuel, E. E., Sherifat, O. A., & Isiaka, A. O. (2011).** Constituents and antimicrobial properties of the leaf essential oil of *Gossypium barbadense* (Linn.). Journal of Medicinal Plants Research, 5(5), 702-705.

[11] **Yadav, R. N. S., & Agarwala, M. (2011).** Phytochemical analysis of some medicinal plants. Journal of phytology, 3(12).

[12] **El-zaidy, M., El-mousalamy, A. M., Hussein, S., & Yunus, M. E. G. (2021).** Phenolics from *Mangifera Indica (Tommy Atkins*) peels and assessment of its Anticancer Activity. Bulletin of Faculty of Science, Zagazig University.

[13] **Olajuyigbe, O. O., & Afolayan, A. J. (2011).** Phenolic content and antioxidant property of the bark extracts of Ziziphus mucronata Willd. subsp. mucronata Willd. BMC Complementary and Alternative medicine, 11(1), 1-8.

[14] **Bauer, A. W. (1966).** Antibiotic susceptibility testing by a standardized single disc method. Am J clin pathol, 45, 149-158.

[15] **Sofowara A. (1993).** In "Medicinal plants and Traditional medicine in Africa". Spectrum Books Ltd, Ibadan, Nigeria. p. 289.

[16] **Bendini A., Cerretani L., Pizzolante L., Toschi T.G., Guzzo F., Ceoldo S., Marconi A., Andreetta F. and Levi M. (2006).** Phenol content related to antioxidant and antimicrobial activities of *Passiflora* spp. extracts. *Eur. Food Res. Technol*., 223: 102-109.

[17] **Cowan, M. M. (1999).** Plant products as antimicrobial agents. Clinical microbiology reviews, 12(4), 564-582.

[18] **Just, M. J., Recio, M. C., Giner, R. M., Cuéllar, M. J., Máñez, S., Bilia, A. R., & Ríos, J. L. (1998).** Anti-inflammatory activity of unusual lupane saponins from *Bupleurum fruticescens*. Planta medica, 64(05), 404-407..U

[19] **Nyarko, A. A., & Addy, M. E. (1990).** Effect of aqueous extract of *Adenia cissampeloides* on blood pressure and serum analytes of hypertensive patients. Phytotherapy Research, 4(1), 25-28.

[20] **Filippo I. (2006).** Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Research Signpost, 37/661 (2): 23-67.