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Antimicrobial and Antioxidant Evaluation of Various Parts of *Gossypium Herbacium* (Linn) Plant

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Abstract

The methanol extracts of the various plant parts were screened for anti-oxidant activity by thin layer chromatography using 2,2-diphenyl-1-dipicrylhydrazyl (DPPH) while in vitro antimicrobial evaluation were determined by agar diffusion method and bioautographic technique using *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as test organisms; aqueous solvent served as control and ciprofloxacin served as standard drug. The result showed that methanol extracts of the stem, leaf, root and seed of the plant were found to be stronger against the entire test organisms and the stem and leaf of the plant were also found to possess strong antioxidant activity. The antimicrobial property shown by the leaf is an evidence of the ethno medicinal uses of the plant. *G. herbaceum* leaf, seed, epicarp, stem bark, root bark, may provide novel plant-derived therapeutic agents, effective in treating infectious diseases arising from multiple drug-resistant bacteria and a target in the management of oxidative stress that could easily lead to aging.

Keywords: Gossypium herbacium; 2; 2-diphenyl-1-dipicrylhydrazyl (DPPH); Eschreichia coli; Bacillus subtilis; Staphylococcus aureus; Pseudomonas aeruginosa; Antimicrobial; Antioxidant activity.

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1. Introduction

Malvaceae is a family of flowering plants containing 243 genera and at least 4,225 species of herbs, shrubs, and trees. Economically, the most important member of the family is Gossypium (cotton) [1]. The genus Gossypium is a leading species, comprises around 50 species in the tribe Gossypioieae and few new species continue to be discovered [2]. The origin of this genus dates back to around 5- 10 million years [3]. The name of the genus is derived from the Arabic word goz, which refers to a soft substance [4]. The word "cotton" originated from the Arabic term 'al qutn', which became in Spanish 'algodón' and cotton in English. Cotton was first domesticated in the Old World about 7,000 years ago [5]. It is native to India, having numerous varieties in this region [6]. A legend was perpetuated from a factual description of *G. herbaceum* plant by Greek historian Herodotus in the 5th century BC. It was first cultivated in China by about 600 AD [7] the plant is mainly grown for its fibers, which are used to make clothing and similar products. Gossypium species are distributed in arid to semiarid regions of the tropics and subtropics. Generally shrubs or shrub-like plants, the species of this genus are extraordinarily diverse in morphology and adaptation, ranging from fire-adapted, herbaceous perennials in Australia to trees in Mexico. Commercial species of cotton plant are *G. hirsutum* (>90% of world production), *G. barbadense*, (3-4%), *G. arboretum* and *G. herbaceum* (together, 2%) [7]. However, *Gossypium herbaceum* has been widely used in the production of food and medicine as well. Cotton seeds are not only a valuable source of vitamins but an excellent pain reliever.

The genus Gossypium of family Malvaceae, consist of about 50 species distributed in arid to semiarid regions of the tropics and subtropics. The source of this genus is about 5-10 million years ago [2]. The 4 domesticated cottons of the world (*Gossypium arboreum* and *Gossypium herbaceum* are the Old World diploids and *Gossypium barbadense* and *Gossypium hirsutum* are the New World tetraploids) have been cultivated separately in various parts of the world [3]. *Gossypium herbaceum* is an Old World cotton plant introduced in the indigenous systems of medicine. This plant has been used in the preparation of medicines and food. Cotton seed is rich source of vitamin E and used as pain reliever, anti-oxidant, laxative. Root bark of this plant used as aphrodisiac and root bark decoction is used for amenorrhea [8]. The part of the plant used in medicine are seeds [9, 10], leaves, [10] root [9], [10], [11] and root bark (Anonymousm2007). In Unani medicine leaves of *Gossypium herbaceum* useful in ishalatfal (childhood diarrhea) and seeds useful in qillatul laban (inadequate lactation). Vernacular names: The plant is known by different vernacular names in the different places by the local people. It is commonly called cotton plant, Levant cotton[12].

2. Materials and Methods

2.1. Plant

All the plant parts materials were collected from a tree located on Obafemi Awolowo University (OAU) campus (Road 1). It was authenticated by comparison with herbarium specimens by the Department of botany, OAU.

2.2. Extraction of Plant Material

The fruits of *G. herbacium* were separated into epicarp and seeds. The epicarp was then macerated (900 g) in methanol (100%) at room temperature for 72 h. The seed (1500 g), stem bark (1100 g), leaf (465 g) and root bark (555 g) were air-dried, powdered and extracted in aqueous methanol at room temperature for 48 h. The mixture of each extraction was then filtered using filter paper. The filtrate was concentrated to dryness in vacuo to yield crude extract *G.herbacium* stem bark (GHSB) (20.06 g), *G.herbacium* root bark (GHRB) (20.28 g), *G.herbacium* leaf (GHL) (30.13 g), *G.herbacium* epicarp (GHE) (10.43 g) and *G.herbacium* seed (GHS) (13.3 5g).

2.3. Antioxidant Screening

Crude extracts of all the parts of the plant were screened for antioxidant activity. The brief description of the procedure is as follows: A solution of the test material in method was spotted on the thin layer chromatographic plate and developed using a suitable solvent system. This was sprayed with DPPH reagent. The chromatogram was exposed to daylight until the purple violet background was bleached. Only zones where the colour turned yellow within the first fifteen minutes after spraying was recorded as positive results (that is, possess antioxidant activity). The result of the screening of the different plant materials are summarized in Table 2.

2.4. Antimicrobial Screening

The agar diffusion (cup plate) method was used for this examination. Molten and cooled agar 60 ml (45°) were separately inoculated with the nutrient broth culture of the test organisms (0.6 ml) and mixed thoroughly. The inoculated medium was then carefully poured into sterile petri dishes (24 cm petri dish) and allowed to set. Thereafter, cups (8 mm diameter) were aseptically bored into the solid nutrient agar using a sterile cork borer. The test solutions 100 ul each were then introduced into each of the cups ensuring that no spillage occurred. Also, the same volume of the standard antimicrobial agent and the solvent were introduced into some of the cups to act as positive and negative controls, respectively. The plates were left at room temperature for 2 h to allow for diffusion into the medium and thereafter incubated face upwards at 37°C for 24 h. Sample was tested in duplicate and diameters of zone of inhibition were measured to the nearest millimetre using transparent ruler [13]. Bioautography technique for anti-microbial screening The method involved an overlay of inoculated agar medium on developed silica gel thin layered chromatography (TLC) glass plate followed by incubation at 37°C for 24 h. Zones of inhibition were detected as clear white areas over a purple background [13].

3. Results

The results of antimicrobial activities Table 1 and antioxidant tests Table 2.

Table-1. Antimicrobial activities of the crude extract of various parts of the plant from *Gossypium Herbacium* in 80mg/ml concentration in methanol: $H_2O(1:1)$

Diameter of zone of Inhibition (mm)					
GHRB	GHL	GHE	GHS	Ciprofloxacin	Methanol
6.5 <u>+</u> 0.02	18.7 + 0.01	6.5+0.01	12.4+0.02	4.5+0.01	-
5.8 <u>+</u> 0.01	10.3 <u>+</u> 0.02	8.5 <u>+</u> 0.01	-	6.5+0.02	-
18.3 <u>+</u> 0.02	18.7 <u>+</u> 0.00	16.5 <u>+</u> 0.02	6.5 <u>+</u> 0.02	15.4+0.01	-
16.5 <u>+</u> 0.01	14.5 <u>+</u> 0.00	13.4 <u>+</u> 0.02	12.4+0.00	4.5+0.01	-
	GHRB 6.5 ± 0.02 5.8 ± 0.01 18.3 ± 0.02 16.5 ± 0.01	GHRB GHL 6.5 ± 0.02 18.7 ± 0.01 5.8 ± 0.01 10.3 ± 0.02 18.3 ± 0.02 18.7 ± 0.00 16.5 ± 0.01 14.5 ± 0.00	GHRB GHL GHE 6.5 ± 0.02 18.7 ± 0.01 6.5 ± 0.01 5.8 ± 0.01 10.3 ± 0.02 8.5 ± 0.01 18.3 ± 0.02 18.7 ± 0.00 16.5 ± 0.02 16.5 ± 0.01 14.5 ± 0.00 13.4 ± 0.02	GHRB GHL GHE GHS 6.5 ± 0.02 18.7 ± 0.01 6.5 ± 0.01 12.4 ± 0.02 5.8 ± 0.01 10.3 ± 0.02 8.5 ± 0.01 $ 18.3\pm0.02$ 18.7 ± 0.00 16.5 ± 0.02 6.5 ± 0.02 16.5 ± 0.01 14.5 ± 0.00 13.4 ± 0.02 12.4 ± 0.00	GHRB GHL GHE GHS Ciprofloxacin 6.5 ± 0.02 18.7 ± 0.01 6.5 ± 0.01 12.4 ± 0.02 4.5 ± 0.01 5.8 ± 0.01 10.3 ± 0.02 8.5 ± 0.01 $ 6.5\pm0.02$ 18.3 ± 0.02 18.7 ± 0.00 16.5 ± 0.02 6.5 ± 0.02 15.4 ± 0.01 16.5 ± 0.01 14.5 ± 0.00 13.4 ± 0.02 12.4 ± 0.00 4.5 ± 0.01

Cup size = 8.0 mm, - = no activity.

Sample	Source	Time taken for colour developement	Antioxidant Activity
GHSB	Stem Bark	Immediate	Strong
GHRB	Root Bark	10 min	Weak
GHL	Leaf	5 min	Strong
GHE	Epicarp	5 min	Weak
GHS	Seed	No reaction	-

Table-2. Antioxidant activities of the crude extracts of various parts of G.herbacium

4. Discussion

The crude extracts of the various parts of *G.herbacium* showed reaction with DPPH after some minutes except the seed. DPPH solution is an established radical solution, known to interact with any substance that is capable of giving hydrogen atom or with another radical present in a radical solvent system. Nitric Oxide (NO), on the other hand, is produced in biological tissues by nitric oxide synthase (NOS), in ideal physiological status in an organism in the process of metabolizing arginine to citrulline through a five-electron oxidative reaction sequence [14]. Overproduction of NO accounts for a compromised structural and functional behavior of several cellular components. Phytochemicals like terpenoids, phenols and flavonoids have been documented to be responsible for

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antioxidative activities in biological systems, scavenging singlet oxygen and free radicals in the system [15-17]. Vitamin E is a fat-soluble compound with unique antioxidant activities. In addition to its antioxidant capacity, it acts in immune function and in the regulation of gene expression and other metabolic processes [18]. It is found in various foods, nuts, seeds, and vegetable oils. Natural vitamin E occurs in eight chemical groups namely; alpha-, beta-, gamma-, and delta-tocotrienol and alpha-, beta-, gamma-, and delta-tocopherol, with different degrees of therapeutic actions [18]. It is, however, worthwhile to note that out of these eight classes of vitamin E, only α tocopherol is known to meet the requirement of human need. Vitamin E as an antioxidant compound is capable of inhibiting reactive oxygen species (ROS). It also shields the biological membrane moiety of the cells from oxidative destruction of reactive free radical molecules. The GHSB showed immediate colour reaction and it was a strong antioxidant activity. The root barks showed a colour reaction after 10 min and it was a weak anti-oxidant activity. The leaf and the epicarp showed colour reactions after 5 min, the leaf had a strong antioxidant activity while the epicarp had a weak anti-oxidant activity. This is suggestive that further work could be done on it to know the active principle that is responsible for the activity. The GHSB and GHL possess strongly anti-oxidant activity after 5 min. This is also suggestive that if further work is done on it extensively the stem bark and the leaf of the plant could serve as a better free radical scavenger and inhibitor of oxidative tissue damage than vitamin C, vitamin E succinate, vitamin C and vitamin E succinate combined, and beta carotene.

In this study, various parts of the crude extracts of *G.herbacium* were subjected to preliminary antimicrobial test. GHSB, GHL and GHS were active against *Escherichia Coli* at a minimum diameter of zone of inhibition ranges from 15.5+0.02 mm to 18.7+0.01 mm. GHSB and GHL were moderately active against *staphylococcus aureus* at a minimum diameter of zone of inhibition ranges from 10.3+0.02 mm to 12.8+0.02 mm. All the various parts of the plant showed both strong and moderate antimicrobial activity against *pseudomonas aeuriginosa* at a minimum diameter of zone of inhibition ranges from 16.4+0.02 mm to 18.7+0.00 mm. It is interesting to note that all the various parts of the plant methanolic crude extract displayed noteworthy inhibitory potentials against *Bacillus subtillis* at a minimum diameter of zone of inhibition ranges from 14.5+0.02 mm to 16.5+0.01 mm. Further work is being done by the author of this paper to isolate the active compounds responsible for both the antimicrobial and antioxidant activity each.

5. Conclusion

The antimicrobial property of the leaf justifies its use in treating diarrhea, scabies, dysentery, gonorrhoea and ophthalmic. This research findings has shown that the stem bark and the leaf possess strong antimicrobial activity and also show a strong antioxidant activity implying that it could also be used to treat ringworm, scabies, dysentery to mention a few and could serve as a free radical scavenger.

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