

ANALYSIS OF ANTIBACTERIAL ACTIVITY OF THE LEAVES OF GOSSYPIUM HIRSUTUM ON SELECTED BACTERIA

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ABSTRACT

Antibacterial and phytochemical analysis of the leaves of Gossypium hirsutum was carried out using selected bacteria; Salmonella typhi, Staphylococcus aureus, Proteus mirabilis and Escherichia coli. The leaves were collected, identified, washed and air dried at room temperature and milled into powder. Forty (40) g of the powder was macerated in extracting solvents (methanol and ethanol) used. The crude extract was concentrated by evaporation at various concentrations (100mg/ml, 300mg/ml, and 500mg/ml) prepared using 5% DMSO. Phytochemical analysis showed that Saponin (+++), Tannin (+++), Alkaloids (+++), and Flavonoids (+) were present. Methanol and ethanol yielded 220mg (0.55%) and 150mg (0.38%) crude extracts respectively. Methanol extract at 500mg/ml exhibited

inhibition zones of 28 mm, 28 mm, 26 mm, and 20 mm against Escherichia coli, Salmonella typhi, Proteus mirabilis and Staphylococcus aureus respectively while ethanolic extract showed inhibition zones of 20 mm, 17 mm, 17 mm, and 7 mm against Escherichia coli, Salmonella typhi, Proteus mirabilis and Staphylococcus aureus. Both extracts showed activity against all the test bacteria except Staphylococcus that was less susceptible to ethanolic extract. This study therefore suggests that methanolic and ethanolic extracts of Gossypium hirsutum have activity against certain bacteria since they exhibited comparable activity with the positive control (ciprofloxacin). Further research should be conducted to isolate, purify and characterize bioactive component of the extracts responsible for the observed antibacterial activity.

KEYWORDS: Antibacterial activity, leaf extract, Gossypium hirsutum, Bacteria.

INTRODUCTION

Scientists from diverse fields are investigating plants with an eye to their antimicrobial usefulness. *Gossypium hirsutum* is one of such plants.

Gossypium hirsutum is one of the genera under the leaf succulent family malvaceae (Duke, 1983). It is a plant that grows up to 10 meters high. It is commonly known as upland plant (Wendel, 2010). *Gossypium hirsutum* bears broad three- segmented greenish leaves which are about 2 inches to 6 inches in length arranged alternately on the stem (Khaleequr, *et. al.*, 2012). The blooms of the plant are cup-shaped with big and flashy petals whose hue ranges from white to yellow (Parnell, 1981). The leaves have a purplish or reddish spot close to their base. They also contain a high concentration of dietary protein, cellulose, glycerin and linolenum (Bijaj, 1998).

Gossypium hirsutum plant has continued to be a major source of drugs and natural products (Newman *et. al.*, 2003). Traditional herbalists in Nigeria use a variety of herbal preparations to treat different kinds of ailments including many microbial infections such as gonorrhoea, sore throat, and gastrointestinal tract infection (Okerulu and Ani, 2001). In most cases, the herbalists are ignorant of the pharmacological and toxicological value of their medications (Egareonu and Mokwe, 2005). Herbal medicine has been shown to be effective and about 80% of the population depends on it for their primary health care (Okwori *et al.*, 2007). This can be attributed to its affordability and accessibility in the economic sense and also due to an uneven distribution of health personnel in the rural areas. *Gossypium hirsutum* has been shown to have strong antifertility, antitumor, antiparasite and anti-HIV properties (Bajaj, 1998).

In recent times attention has been directed to medical research to substantiate the claims made by traditional healers and provide scientific basis for their efficiency (Sofowora, 1982; Newman *et. al.*, 2003). Most studies on Nigerian medicinal plants have emphasized the antimicrobial activities of individual plants (Okerulu and Ani, 2001).

The use of plants for healing is as ancient and universal as medicine itself and many infections are known to be treated with herbal remedies throughout the history of mankind (Egareonu and Mokwe, 2005). Plants act generally to stimulate and supplement the body's healing forces; they are the natural food for human beings (Newman *et. al.*, 2003).

The leaf of *Gossypium hirsutum* has been reported to be used for the management of menstrual issues and the roots for treating syphilis, boils, ulcers and septic swelling. Methanoic crude extract has been found active against standard strains of *Aspergillus niger*, *Lieotricum candidum* and *Candida utilis*.

Antibiotic resistance which is basically the ability of micro-organisms to survive exposure to an antibiotic is primarily as a result of genetic mutations and the high this phenomenon could be attributed to antibiotic use and misuse in health facilities and communities (Martinez and Baquero, 2000).

In this study the phytochemical and antibacterial screening of leaf extracts of *Gossypium hirsutum* was carried out with a view to identify phytochemical components and the effect on the extract on bacterial growth.

OBJECTIVES

- i. To carry out methanol and ethanol extraction of *Gossypium hirsutum* leaf.
- ii. To determine the photochemistry of the plant
- iii. To carrying out *in-vitro* antibacterial susceptibility test on selected bacteria using methanol and ethanol crude extract of *Gossypium hirsutum* leaf.
- iv. To compare the activity of methanol with ethanol crude extract of *Gossypium hirsutum* leaf.

MATERIALS AND METHODS

Collection of plant samples

Fresh leaves of *Gossypium hirsutum* was collected from Nkwere in Oyi Local Government Area of Anambra State. The leaf was identified by a plant taxonomist Prof J.C Okafor of Enugu State University of Science and Technology.

Processing of plant sample

Fresh leaves of *Gossypium hirsutum* were harvested and sorted. Yellowish and dried leaves were removed and only the green ones without spot used. The leaves were washed with distilled water, spread on local tray made of palm fronts so that the water could drain off and kept inside a well ventilated room where they were turned after every 6hrs to dry. The dried leaves were ground using sterile laboratory pestle and mortar and the powder stored in an air tight sterile container until used for analyses.

Extraction (using maceration method)

The solvents- methanol and ethanol (250ml) were measured into conical flasks containing 40g of the powder respectively, covered with a foil, agitated vigorously every 6 hour for 24 hrs. The mixture was filtered after 24 hrs using muslin cloth and the filtrate concentrated by evaporation in a water bath at 37°C for 1 hr.

Test bacteria

The stock clinical isolate of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Proteus mirabilis* were obtained from the Department of Microbiology and Biotechnology Caritas University Enugu.

Bacterial identification

The stock bacteria were identified using standard bacterial identification techniques (Gram staining, motility and biochemical tests).

Preparation of media Nutrient Agar

Nutrient agar for susceptibility test was prepared following manufacturers instruction.

Preparation of different concentration of the extract

100mg/1ml, 300mg/ml and 500mg/ml concentrations of the crude extracts of methanol and ethanol were prepared by dissolving 1g, 3g and 5g of the extract in 10m 5%DMSO respectively.

Bacterial susceptibility testing

Agar Diffusion Test was employed in testing susceptibility of bacterial to the extracts. The prepared nutrient agar plates were incubated for 24 hrs to test for sterility before use. The test organisms were streaked on the surface of the agar and with the aid of a sterile cork borer, 3 wells were bored equidistant to a well at the centre which is the control (Antibiotics). A drop of each concentration of the extracts was aseptically introduced into wells 1-3, while the control at the centre. The plates were incubated for 24 hours at 37°C and zones of inhibition were measured using a ruler calibrated in millimeters.

Phytochemical screening

Phytochemical screening of the leaf was carried out to test for Tannins, saponins, glycosides, alkaloids, flavonoids and sterols (Trease and Evans, 1988).

Test for Tannins

Two drops of 5% FeCl₃ was added to 1ml of the extract. A blue —black or blue green precipitate was taken as evidence for the presence of tannins.

Test for glycosides

About 2.5ml of dilute H₂SO₄ acid was added to 5ml of the extract in a test tube, boiled in a water bath for 15mins, cooled and neutralized with 20% KOH solution. Five (5) ml of a mixture of Fehling's solution A and B was added and boiled. A brick-red precipitate shows reducing sugars released as a result of the hydrolysis.

Test for the presence of saponins

Half (1/2) gram of the powder was boiled with 10ml of distilled water for 5minutes. The mixture was filtered while still hot. The filtrate was then used for the following tests.

• Emulsion test

One (1) ml of the filtrate was added to 2 drops of olive oil. The mixture was shaken and observed for the formation of emulsion.

• Frothing test

One (1) ml of the filtrate was diluted with 4ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable froth.

Test for the presence of flavonoids

Half (1/2) gram of the powder was heated with 10ml of ethylacetate in a water bath for 3 minutes. The mixture was filtered and the filtrate used for the following tests:

- Four (4) ml of the filtrate was shaken with 1ml of 1% aluminum chloride solution and observed for light yellow colouration in the ethyl acetate layer. A yellow colouration in the ethylacetate layer indicates the presence of flavonoids.
- Four (4) ml of the filtrate was shaken with 1ml diluted ammonia. The layers were allowed to separate. A yellow colouration at the ammonia layer indicates the presence of flavonoids.

Test for alkaloids

One (1) gram of the leaf extract of *G. hirsutum* was boiled with 25ml of 2% HCl on a water bath for 5 minutes. The cooled mixture was filtered and 1ml portion of the filtrate treated with 2 drops of the following reagents:

- Dragendorff's reagent (Bismuth in potassium iodide solution). A red precipitate indicates the presence of alkaloid.
- Mayer's reagent (potassium in mercuric iodide solution). A creamy white coloured precipitate indicates the presence of alkaloids.
- Wagner's reagent (iodine in potassium iodide solution). A reddish brown coloured precipitate indicates the presence of alkaloid.

RESULTS

Extract Yield

Table 1: Extract yield using 250ml solvent/40g of the powder.

S/N	Extracting solvent	Yield (%)
1	Methanol	220mg (0.55)
2	Ethanol	150mg (0.38)

Methanol extracted the largest quantity (220mg) of materials from the leaf with 220 mg yield followed by ethanol with 150mg yield.

Phytochemical analysis

Phytochemical analysis of the leaves of *Gossypium hirsutum* showed that saponins, tannins, alkaloids and flavonoids were present, while steroids was absent as presented in table 2.

Table 2: Phytochemical of *Gossypium hirsutum* leaves.

Photochemical	Estimated concentration
Alkaloids	+++
Flavonoids	+
Saponins	+++
Glycosides	++
Steroids	-
Tannins	+++

Key:

-	-	Absent
+	-	Scanty
++	-	Moderate
+++	-	Abundant

Table 3: Effect of various concentrations of Methanol Extract on the test organisms.

S/N	Extract conc. (mg/ml)	Zone of Inhibition (mm)			
		ST	PM	SA	EC
1.	500	28	26	10	28
2.	300	15	19	07	24
3.	100	0	08	04	18
4.	Control Sterile disc	-	-	-	-
	Ciprofloxacin (10)	30	28	24	30

Key:

ST: *Salmonella typhi*

PM: *Proteus mirabilis*

SA: *Staphylococcus aureus*

EC: *Escherichia coli*

Controls

Negative – Sterile sensitivity disc

Positive – 10g/ml Ciprofloxacin

Table 4: Effect of various concentrations of Ethanol Extract on the test organisms.

S/N	Extract conc. (mg/ml)	Zone of Inhibition (mm)			
		ST	PM	SA	EC
1.	500	17	17	07	20
2.	300	15	15	05	10
3.	100	11	11	03	08
4.	Control Sterile disc	-	-	-	-
	Ciprofloxacin (10)	30	28	24	30

Key:

ST: *Salmonella typhi*

PM: *Proteus mirabilis*

SA: *Staphylococcus aureus*

EC: *Escherichia coli*

Controls

Negative – Sterile sensitivity disc

Positive – 10g/ml Ciprofloxacin

DISCUSSION

The result of extract yield using methanol and ethanol revealed that methanol extracted the largest quantity (220mg) with 0.55% yield followed by methanol (150mg) with 0.38% yield.

This is using a constant quantity/weight of the powdered extract and equal volume of the extracting solvents. Thus when quantity of materials extracted is of important methanol should be used instead of ethanol. The observed higher zone of inhibition observed against test bacteria using methanol may be explained by the ability of the solvent to extract more bioactive agent than ethanol. This finding agreed with that reported by Sasidharam *et. al.* (2010) in a study to extract, isolate and characterize bioactive compounds from plants' extract. Also, Akinyemi *et. al.* (2005) while screening crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicilin resistant *Staphylococcus aureus* activity reported higher yield using methanol.

Phytochemical analysis of the leaves *Gossypium hirsutum* using different extracting solvents showed that saponins, tannins and alkaloids were abundant (+++), glycosides were moderate (++) , flavonoids were scanty (+), while steroids were absent (-). This is an agreement with the finding of Gupta and Sharma (2006) in a review on medicinal plants exhibiting anti-fertility activity in males.

As such the observed antimicrobial effects of the various extracts may be attributed to single or combined effect of the bioactive constituents in the leaf. Comparing the activity of methanol extract on *Salmonella typhi*, *Staphylococcus aureus*, *Proteus mirabilis* and *Escherichia coli*, it was found that *Salmonella typhi* and *Escherichia coli* were more susceptible to the extract followed by *Proteus mirabilis*, while *Staphylococcus aureus* appeared not to be susceptible to methanol extract. Thus even at 100 mg/ml which was the least concentration used, the three organisms were susceptible. This finding suggests that methanol extracted the most bioactive components of the plant. Similar pattern was observed using ethanol extract. The extract at 300mg/ml and 100mg/ml were sensitive as zones of inhibition were more than 10mm, except for *Escherichia coli* and *Staphylococcus aureus* that recorded zones of inhibition of 8mm and 3mm respectively. This was unlike methanol extract which had activity on the four test organisms at all concentrations with the exception of *Staphylococcus aureus*. However, activity was found to decrease with decrease in concentration of the extract.

This research work showed that methanol and ethanol extract was active against *Salmonella typhi*, *Proteus mirabilis* and *Escherichia coli* but not *Staphylococcus aureus* at the concentrations of 500mg/ml and 300mg/ml. this further may explain while leaf extract of this

plant is used by local folks in treating typhoid fever, urinary and gastrointestinal tract infections.

CONCLUSION

Gossypium hirsutum have been discovered to contain phytochemical substances that are bioactive against wide range of bacteria. Solvent used in extraction plays vital role in the activity of the extract *in-vivo* and *in-vitro*. Further research should be conducted on animals and humans to determine the effectiveness and impact *in-vivo* of these extracts *in-vivo*.

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