

Antibacterial and Phytochemical Analysis of Garden Egg (*Solanum aethiopicum*) Leaf on Bacterial Isolates Among Patients with Conjunctivitis

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ABSTRACT

Background: Resistance of microorganisms to conventional antibiotic therapy have become a major global problem and a threat to the efficacious treatment of infectious diseases that affect the body. Medicinal Plants have been reported as Promising Sources of antibacterial drugs due to their potent biochemical active compounds. This study was carried out to identify the phytochemicals and assess the antibacterial activity of *Solanum aethiopicum* leaf extracts for potential treatment of systemic and ocular bacterial infections.

Methods: This was a clinical study carried out using the convenient sampling method at the molecular laboratory and Department of Optometry Teaching clinic of the Federal University of Technology Owerri, Imo State, Nigeria.

Ethanol and Aqueous extracts of *Solanum aethiopicum* leaf were prepared using Soxhlet extraction and cold maceration methods respectively. Quantitative and qualitative phytochemical analysis of the extracts were carried out. Swabs were taken from the Conjunctiva of 20 subjects with infected eye. Bacteria species of gram positive and negative stains were isolated and cultured. The organisms were identified using the agar-well diffusion method. The leaf extracts were prepared at different concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. the antibacterial activities of the leaf extracts were tested against gram positive isolates which included *Staphylococcus aureus*, *Streptococcus pyogenes*, *Micrococcus luteus*, *Bacilli spp.* and the gram-negative isolates, namely, *Klebsiella pneumoniae*, *Shigella flexneri*, *Enterobacter cloacae*, and *Escherichia coli*. The zones of inhibition of the microbes were measured. The minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) were determined. Ciprofloxacin, a standard antibiotic, was used as a positive control to determine the potency of the leaf extracts. Data was analyzed using t-test and ANOVA.

Results: The phytochemical analysis revealed that the ethanol extract of *Solanum aethiopicum* contains greater content of bioactive ingredients in the order, flavonoid, saponin, oxalate, steroid, cardiac glycosides, hydrogen cyanide, tannin, alkaloid, Phytate and phenol. All tested isolate showed significant antibacterial activity with p value = 0.001. There was no significant difference in the antibacterial activity of ethanol and aqueous extracts P value= 0.056. There

was a significant difference between the leaf extracts (ethanol and aqueous) and the positive control (Ciprofloxacin) P value 0.0001. On multiple comparison of the antibacterial activities of the ethanol extract, aqueous extract and the positive control on the gram positive and the gram-negative bacteria isolates, the gram-positive bacteria isolates were found to be more susceptible than the gram-negative bacteria stains P value = 0.001. Minimum inhibitory concentrations were significant across the aqueous extract, ethanol extract and positive control P value= 0.0001, with mean value 100mg/dl, 10.2mg/dl and 6.25mg/dl respectively.

Discussion: The above results has revealed the order of superiority in the antibacterial efficacies of the extracts of *Solanum aethiopicum* and the control antibiotics; highlighting the ciprofloxacin as the highest, followed by the ethanol and aqueous extract with significant susceptibility of the gram-positive strains than the negative bacterial strains.

Conclusion: The study concluded that the ethanoic and aqueous leaf extracts of *Solanum aethiopicum* could serve as a potent broad spectrum antibacterial agent for systemic and ocular bacterial infection.

Recommendation: Studies should be carried out to assess other parts of the plant model.

Keyword: *Solanum aethiopicum*, Minimum inhibitory concentration, Minimum bacteria concentration.

INTRODUCTION

Ocular infections are preventable causes of vision loss worldwide. These infections are due to varieties of pathogens which includes, bacteria, viruses, fungi and parasites.^{1, 2} The Conjunctiva is a thin translucent mucous membrane which lines the posterior surface of the lid and the anterior surface of the eyeball. Conjunctivitis is defined as the inflammation and swelling of the conjunctiva tissue with subsequent blood vessel engorgement.^{3, 4, 5} Bacteria causes less than 20% of acute cases of infectious conjunctivitis in adults and 70% in children.⁶ Conjunctivitis due to infections are frequent cases seen by Optometrists and Ophthalmologists accounting for one-third of eye related emergency visit.^{7, 5.}

Staphylococcus aureus, *streptococcus pneumoniae*, *pseudomonas aeruginosa* are leading isolates in ocular infection.⁵ *Staphylococci*, *streptococcus pyogenes* and *pseudomonas aeruginosa* are common in blepharitis; *staphylococci*, *streptococcus pneumoniae*, *pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* in conjunctivitis; *staphylococci*, *P. aeruginosa*, and *E.coli* in Dacryocystitis, Coagulase negative *staphylococci*, *pseudomonas aeruginosa* and *staphylococcus aureus* in *keratitis*,

Streptococcus viridans, *Streptococcus pneumoniae* and coagulase negative *staphylococci* in endophthalmitis, *Klebsiella pneumoniae*, *Bacillus specie* and *Coagulase negative* are common causes of post traumatic infections.^{8, 9} Some cases of bacterial conjunctivitis are self-limiting between the periods of 7 to 10 days.⁹ Antibiotic therapy is the primary approach to treating bacterial conjunctivitis. Resistance of microorganisms to conventional antibiotic therapy is a major global problem and quietly threatens treatment outcome of infectious diseases.^{10, 11} The Antibiotic Resistance Threat Report from the Centers for Disease Control and Prevention, United States experiences over 2.8 million antibiotic-resistant infections annually, leading to more than 35,000 death.¹² There is need for new or comorbid measures to combat bacterial infections.

Plants are multicellular organisms in the kingdom (Plantae) that use the process of photosynthesis to make their food. A medicinal plant is one that contains substances that can be used for therapeutic purposes in health care.⁶ Apart from the medicinal role of natural plants, they are also source of new drugs.¹³ About 50% of all modern pharmaceutical drugs can be traced back to traditional, ethnobotanical source of

medicine.¹⁴ Biochemical compounds found in plants; the phenolic compounds, Alkaloids, Saponins and Terpenoids have exhibited noteworthy antibacterial potential. Mechanism of action is through membrane disruption mechanism, protein binding, interference with intermediary metabolism, anti-quorum sensing, and anti-biofilm activity, development of reactive oxygen species.¹⁵ Given the widespread use of these plants, it is important for healthcare practitioners to be informed about herbal antibiotics as an adjunct or alternative therapy.

Garden egg (*Solanum aethiopicum*) plant is found in Asia and Topical Africa. It is a vegetable crop mainly grown for its fruits and leaf. It is used in the preparation of vegetable stew, soup and yam dishes. It can be eaten raw or used to prepare salad. *Solanum aethiopicum* leaf, commonly known as Garden egg leaf in English and Anara leaf in Igbo is medicinal, a natural blood tonic.⁷ It is also widely used to treat infections due to its antimicrobial, anti-inflammatory, and antioxidant activities. The study seeks to identify the biochemical compounds of the leaf and its broad-spectrum activity against a broad range of ocular bacteria.

MATERIALS AND METHODS

The research was a clinical study involving 20 subjects presented to the Optometric Teaching Clinic, Federal University of Technology Owerri between December 2024 and January 2025. The convenient sampling method was used to obtain the subjects. Informed consent was obtained from subjects who participated in the study. Ethical clearance was obtained from the ethics committee of the school of health technology. Case history, visual acuity, external and internal eye examinations were conducted to establish subjects with eye infections. Conjunctiva swabs were collected and taken to the laboratory for culture and identification of offending bacteria organisms. Bacteria species of gram positive and negative stains were isolated.

The organisms were identified using the agar-well diffusion method. Ethanolic and Aqueous extracts of *Solanum aethiopicum* leaf were prepared using Soxhlet extraction and cold maceration methods respectively.¹⁶ Quantitative and qualitative phytochemical analysis of the extracts were carried out. The leaf extracts were prepared at different concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml.

The antibacterial activities of the leaf extracts were tested against gram positive isolates which included *Staphylococcus aureus*, *Streptococcus pyogenes*, *Micrococcus luteus*, *Bacilli spp.* and the gram-negative isolates, namely, *Klebsiella pneumoniae*, *Shigella flexneri*, *Enterobacter cloacae*, and *Escherichia coli*. The zones of inhibition of the microbes were measured. The minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) were determined. Ciprofloxacin, a standard antibiotic, was used as a positive control to determine the potency of the leaf extracts. Data was analyzed using t-test and ANOVA.

PHYTOCHEMICAL ANALYSIS

Qualitative analyses were carried out using the methods of Trease and Evans¹⁷ to ascertain the presence of the different phytochemicals in the leaf.

NUTRIENT AGAR PREPARATION

Preparation of nutrient agar was done by weighing the accurate quantity of nutrient agar in grams and dissolve in distilled water. The mixture was poured into the conical flask and corked and auto cleaved for 15minutes at 121C to form a homogenous solution. It was cooled and poured into sterilized petri-dishes to solidify.

PREPARATION AND STANDARDIZATION OF TEST MICROORGANISMS

The bacteria isolates were inoculated in the petri-dishes using sterile wire loop and the spread to enable them grow in the nutrient agar at 37C for 24 hours. The test organisms were picked up by a sterile loop from the

culture and was transferred and suspended into a tube containing sterile normal saline (NACL, 8.5g distilled water IL) this was then placed in an incubator for 5-10 minute until it achieved turbidity by Mc standard.

TEST FOR ANTIBACTERIAL ACTIVITY

Agar-well diffusion method by Valgas was used.¹⁸ About 0.1ml of the standardized 24-hour old culture of the tested organisms in nutrients broth were spread unto sterile prepared nutrient agar plates, wells of 6mm in diameter was bored on the plates. *Solanum aethiopicum* extracts were poured into these holes and allowed for 30mins. These was incubated at 37C for 24 hours. Inhibition zones were formed on the agar and was measured to the nearest millimeter.

MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION

The following concentrations (6.25%, 12.5%, 25%, 50%, and 100%) of extracts were prepared. Exactly 100ul of each standardized test organism was introduced into the tube of extract. A tube containing only nutrient broth and bacteria without extract served as negative control while another tube containing just the extract and broth without bacteria served as positive control. Each tube was incubated for 18-24 hours then examined for visible growth or turbidity. The concentration of the extract in the tube in which no visible growth was observed and compared with the controls was taken as the MIC.

MINIMUM BACTERIAL CONCENTRATION DETERMINATION

A small aliquot, and 0.1ml was taken from the test tubes (minimum inhibitory concentration) and transferred to new tubes containing fresh nutrient broth and incubated for 37 degree centigrade for 24 hours. Each tube was checked for visible growth or not (turbidity). This was measured and recorded as the minimum bacterial concentration.

ANTIBIOTIC SENSITIVITY TESTING.

Agar well diffusion method by Baulouiri was used.¹⁹ The test organism was seeded on Muller-Hinton agar. Oral ciprofloxacin (500mg) tablet at varying concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, and 6.25mg/ml) was embedded in sterile paper discs. The embedded discs were carefully placed on agar plates inoculated with bacterial cultures with a sterile forcep. The set-up was incubated aerobically at 37 degrees centigrade for 24 hours. The inhibition zones were measured with meter rule and recorded in millimeters.

PROCEDURE FOR DATA ANALYSIS

Data collected from this study was uploaded on the Statistical Package for Social Science (SPSS) version 23 to determine the statistical values of the data. A one sample T-test was used to test the hypothesis of this work at 95% confidence and 0.05 level of significance and 95% confidence interval.

RESULTS

Table 1: Phytochemical analysis, revealing the bioactive compounds found in *Solanum aethiopicum* leaf revealing that ethanoic extract yielded greater amounts of active ingredient of *Solanum aethiopicum* which included; flavonoid, Saponin, oxalates and Steroid.

Parameters	Aqueous extract	Ethanol extract
Flavonoid, µg/g	88.24	1408.82
Saponin, µg/g	1932.63	14972.63
Terpernoid, mg/100g	0.48	0.88
Phenol, mg/g	0.10	1.40
Alkaloids, µg/g	1.02	14.85
Cardiac Glycosides, %	4.14	3.58
Tannins, %	1.40	71.65
Steroids, mg/100g	4.13	21.52

Hydrogen cyanide, mg/kg	1.57	0.15
Oxalate, g/100 g	112.50	112.50
Phytate, %	0.34	0.55

Table 2: Antibacterial activity of aqueous extract on test organisms. All tested isolate showed significant antibacterial activity with P value = (0.001).

Sample	Test Organism	Mean	Std. Deviation	T-test value	P-value
Aqueous Gram +VE	Bacillus Spp	8.5000	5.01664	5.358	0.001
	Staphylococcus Aureus	4.5000	1.77281	7.180	0.001
	Micrococcus luteus	17.0000	4.44722	6.772	0.001
	Streptococcus Pyogene	8.3000	5.53875	4.739	0.001
Aqueous Gram -VE	Klebsiella Pneumoniae	6.1000	2.42441	7.957	0.001
	Enterobacter Cloacae	6.7000	3.12872	6.772	0.001
	Shigella Flexneri	5.0000	.89443	13.693	0.001
	E. Coli	3.4000	1.71270	6.278	0.001

Table 3: Antibacterial activity of ethanol extract on test organisms.

Sample	Test Organism	Mean	Std. Deviation	T-test value	P-value
Ethanol Gram +VE	Bacillus Spp	9.6000	1.83787	16.518	0.001
	Staphylococcus Aureus	4.7000	1.70294	8.728	0.001
	Micrococcus Luteus	17.2000	4.44222	12.244	0.001
	Streptococcus Pneumoniae	11.3000	5.94512	6.011	0.001
Ethanol Gram -VE	Klebsiella Pneumoniae	6.7000	2.45176	8.642	0.001
	Enterobacter Cloacae	9.7000	3.09300	9.917	0.001
	Shigella Flexneri	9.2000	2.85968	10.173	0.001
	E. Coli	5.5000	1.35401	12.845	0.001

All tested isolate showed significant antibacterial activity with P value = (0.001).

Table 4: Shows that there no significant difference in the antibacterial activity of the ethanol and aqueous extracts of the leaf with P- value = 0.056.

Sample solution	Mean	Standard deviation	T-test value	P-value
Ethanol	9.2375	4.86851	1.928	0.056
Aqueous	7.6486	5.35694		

Table 5: Shows a significant difference in the antibacterial activity between the extracts of the leaf and the control antibiotics P value = 0.0001. The control antibiotics has marked antibacterial activity on the bacteria isolates than ethanol and aqueous extract.

Extracts	N	Mean	Standard Deviation.	F-ratio	P-value	Post-hoc test Subset for alpha = 0.05	
						Subset 1	Subset 2
Ethanol	80	9.2375	4.86851	126.009	0.0001	9.2375	21.787
Aqueous	80	7.6486	5.35694			7.6486	
Antibiotics	80	21.787	7.6929				

Table 6: The antibacterial effect of ethanol and aqueous extracts of garden egg leaf and the control antibiotics are much higher for the gram (+) bacteria than the gram (-) bacteria with P value =<0.005, revealing that the ethanol extract, aqueous extract and the control antibiotics had higher antibacterial activities on the gram-positive bacteria than the gram-negative bacteria.

Sample solution	Bacteria type	Mean	Standard Deviation	T-value	P-value
Ethanol	Gram +	10.700	5.8843	2.801	0.006
	Gram -	7.7750	2.9998		
Aqueous	Gram +	9.8421	6.3353	3.967	0.001
	Gram -	5.3333	2.5856		
Control	Gram +	24.875	7.7399	3.899	0.001
	Gram -	18.700	6.3577		

Table 7: Effect of the minimum inhibitory concentration of aqueous extract, ethanol extract and the positive control on bacteria isolates showing significant difference in the antibacterial activity (MIC) between the extracts of the leaf and the control antibiotics with P value = 0.0001. Control antibiotics is the highest in significance followed by ethanol and aqueous extract.

	N	Mean	Standard Deviation	F-ratio	P-value	Post-hoc test Subset for alpha = 0.05		
						Subset 1	Subset 2	Subset 3
Ethanol	8	10.1563	3.23468	6451.667	0.0001	10.1563	100.00	6.2500
Aqueous	8	100.0000	0.0000					
Control	8	6.2500	0.0000					

DISCUSSION OF FINDINGS

This research has brought to light the nutritional and medicinal properties of *Solanum aethiopicum* leaf. The ethanolic and aqueous extracts of *Solanum aethiopicum* leaf contains several bioactive compounds with antibacterial activity against the isolated bacterial strains used in this study. The phytochemical analysis of both the ethanolic and aqueous leaf extracts revealed the presence of Flavonoid, Terpenoid, Phenol, alkaloid, Saponin, Cardiac glycosides, Tannin, Steroids, Hydrogen Cyanide, Oxalate and Phytate. *Solanum aethiopicum* leaf is very rich in flavonoids, saponins, oxalates and steroids. The extracts (ethanolic and aqueous) of *Solanum aethiopicum* leaf exhibited significant antibacterial effects on each bacterium isolate with P-value = 0.0001. On comparing the effect of ethanolic and aqueous extract on the general spectrum of organisms, revealed no significant difference between both extracts P= 0.056. This could be due to the similar phytochemical profiles in both aqueous and ethanol extracts of the leaf. The control antibiotics gave marked significant difference in antibacterial activity against both extract (ethanol and aqueous) P value = 0.0001 revealing its broad spectrum of activity, high level of organism susceptibility and its current use as conventional treatment of bacterial infections. The antibacterial effect (zone of inhibition) across the extracts (aqueous, ethanol) and the control antibiotics was statistically significant for gram positive organisms as against the gram-negative organisms revealing their high degree of susceptibility of the gram-positive strain to leaf extract and the control. The mean minimum inhibitory concentration between

aqueous extract, ethanol extract and the control antibiotics were of high significance, the aqueous = 100mg/dl, ethanol = 10.2mg/dl and the control antibiotics = 6.25mg/dl with P value = 0.0001 (multiple comparison). Their respective values also denote the order of superiority in antibacterial efficacy, with the control antibiotics having the most significant antibacterial activity, followed by the ethanol extract and the aqueous extract.

CONCLUSION

This study reveals the therapeutic potential of *Solanum aethiopicum* leaf as a potent and alternative source of antibacterial agent in treating bacterial infections including those of ocular origin.

Declaration by Authors

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