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A Comparative Analysis of the Antibacterial Activities of Aqueous and Ethanol Extract of Garden Egg Leaf on Ocular Bacterial Isolates

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ABSTRACT

Background: Several reports have it that the bioactive compounds found in plants are effective antimicrobials and good source of antibacterial drugs. This study assesses the antibacterial activities of Ethanol and Aqueous extract of Garden egg leaf for potential treatment of bacterial infections.

Methods: An invitro clinical study carried out at the molecular laboratory using ocular infected conjunctiva swabs of 20 walk in patients at the Department of Optometry Teaching clinic of the Federal University of Technology Owerri, Imo State, and using convenient sampling method. Ethanolic and Aqueous extracts of garden egg leaf were prepared using Soxhlet extraction and cold maceration methods respectively. Bacteria species of gram positive and negative stains were 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 pyogene*, *Micrococcus luteus, Bacilli spp* and the gram-negative isolates, namely, *Klebsiella pneumoneae, Shigella flexneri*, *Enterobacter clocae, and Escherichia coli*. The zones of inhibition of the microbes were measured. 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: Each tested isolate showed significant susceptibility to both ethanol and aqueous extract with p value = 0.001. However, on comparing the antibacterial activity of aqueous and ethanol extract on all tested organisms revealed no significant difference with P value= 0.056. There was a significant difference between the leaf extracts (ethanol and aqueous) and the positive control (Ciprofloxacin) with P value 0.0001.

Discussion: The aqueous and ethanolic extracts of garden egg leaf revealed significant antibacterial activity but not to be compared with the control antibiotics (Ciprofloxacin) which showed superiority.

Conclusion: The study concluded that the aqueous and ethanol extracts of garden egg leaf are potent broad spectrum antibacterial agent for systemic and ocular bacterial infections.

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

Keyword: Zone of inhibition, aqueous extract, ethanol extract, ciprofloxacin antibiotics

INTRODUCTION

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 and widely used to treat infections due to its antimicrobial, anti-inflammatory, and antioxidant activities.[1, 2, 3] Eye infections are preventable causes of vision loss worldwide. These infections are due to varieties of pathogens which includes, bacteria, viruses, fungi and parasites.[4,] Conjunctivitis is defined as the inflammation and swelling of the conjunctiva tissue with subsequent blood vessel engorgement.[5, 6, 7] Bacteria conjunctivitis causes less than 20% of acute cases in adults and 70% in children.[6] *Staphylococcus aureus, streptococcus pneumoniae, pseudomonas aeruginosa* are leading isolates in ocular infection.[7] *Staphylococci, streptococcus pyogene*s and *pseudomonas aeruginosa* are common in blepharitis; *staphylococci, streptococcus pneumoniae, pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Escherichia coli* in conjunctivitis; staphylococci, P.aureuginosa, and E.coli in Dacryocystisis, 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, 10] 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 has become a threat to treatment outcome, hence this study. [11]

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. [12] The phytochemical analysis of the aqueous and ethanol extracts was done using Trease and Evans method[13]. 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 pyogene*, *Micrococcus luteus, Bacilli spp* and the gram-negative isolates, namely, *Klebsiella pneumoneae, Shigella flexneri*, *Enterobacter clocae, and Escherichia coli*. The zones of inhibition of the microbes were measured. 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.

AQUEOUS EXTRACTION OF GARDEN EGG LEAF: COLD MACERATION

50grams of leaf powdered sample was weighed into a sterile 250ml conical flask. 100ml of sterile distilled water was poured into the powder, corked and allowed to soak for 24 hours. The mixture was filtered off after 24hours using sterile filter cloth. The leaf extract was allowed to evaporate using rotary vacuum evaporator at 60 degree centigrade to remain just the extract.

SOXHLET EXTRACTION OF GARDENEGG LEAF USING ETHANOL AS SOLVENT

100ml of 95% ethanol was poured into a soxhlet flask. 50gram of the leaf powder (sample) was placed into the extractor. The heating mantle was set at the boiling point of the solvent (78 degree centigrade). The extract seeped through the reflux arm and back down into the soxhlet flask. This was allowed to continue to circulate until the completion of the extraction. The extract was removed from the siphon tube; the ethanol was evaporated using rotary vacuum evaporator at 50-degree centigrade leaving only the leaf extract.[12]

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.[14] 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.

ANTIBIOTIC SENSITIVITY TESTING.

Agar well diffusion method by Baulouiri was used.[15]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 was 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: 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 2: 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 .3. 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 4. 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 5. 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 + Gram -	10.700 7.7750	5.8843 2.9998	2.801	0.006
Aqueous	Gram + Gram -	9.8421 5.3333	6.3353 2.5856	3.967	0.001
Control	Gram + Gram -	24.875 18.700	7.7399 6.3577	3.899	0.001

DISCUSSION OF FINDINGS

This research has brought to light the medicinal properties of Garden egg leaf. The bioactive compounds of ethanoic and aqueous extracts of garden egg leaf exhibited antibacterial activity against the isolated bacterial strains used in this study. The extracts (ethanolic and aqueous) of garden

egg leaf showed 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. 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.

CONCLUSION

This study reveals the therapeutic potential of garden egg 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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