

Assessment Of Some Plant-Based Traditional Eye Medicinal Herbs For Bacterial Ocular Infectious Treatments

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Abstract

Background: In developing countries, medicinal herbs are used in the treatment of ocular diseases. In the current study, the effects of selected herbs used in the treatment of eye diseases were tested on isolated bacterial ocular infections.

Materials and methods: The bacterial ocular infections were obtained from patients attending Abia State University, Uturu Optometry Clinic. Tears from the patients were collected for culture using Schirmer strips. Isolates were identified by colonial morphology, microscopy and biochemical tests. The medicinal plants analyzed included *Bryophyllum pinnatum*, *Occimum gratissimum* and *Aspillia africana*. A quantitative analysis was conducted to evaluate the phytochemical composition of the selected plants.

Results: The results showed that out of 102 males, 17 (16.66%) showed microbial contamination of the eyes, while 74 (26.90%) females showed similar contamination. The most affected group was the farmers (36.58%), followed by the unemployed (29.41%), and with the highest infection rate observed in the age group 61+ (41.44%) followed by the age group 31-35 (32.25%). The screened individuals reported ocular itching (31.57%), and reddened eyes (27.69%). The most commonly identified bacterial species was *S. aureus*, followed by *Escherichia coli*. Phytochemical analysis revealed that the flavonoids and alkaloids had the highest concentrations in each plant. *Bryophyllum pinnatum* showed the strongest inhibitory effect, followed by *Occimum gratissimum*, while *Aspillia africana* showed the least inhibitory effect.

Conclusion: This study shows that the selected plants have the potential to be used in the treatment of ocular diseases caused by verifying bacterial infections.

Keywords: Eye, Ocular diseases, bacterial infections, herbs, treatment eye diseases

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I. Introduction

The eye is a very important organ of the human body, entrusted with the sensitive task of seeing and several other secondary functions. It is a delicately designed organ that gives comfort and meaning to our movements and therefore plays a very important role in our daily activities. Visual impairment is therefore a major health problem all over the world. About 90% of visually impaired people in the world live in developing countries¹. Interestingly, most cases of visual impairment are preventable. World Health Organization (WHO) statistics show that 285 million people worldwide are visually impaired, of which 39 million are blind and 246 million have low vision. In Nigeria, 1.13 million people aged 40 years and above are blind. Two million, seven hundred (2.7 million) have moderate visual impairment and another 400,000 have severe visual impairment².

The impact of visual impairment is enormous. The most outstanding is the sedentary life of the visually impaired and the total dependence on guides to take them around. The means of livelihood and employability are reduced to the lowest level. The affected person becomes a burden on other relatives. In addition, the person becomes emotionally and psychologically depressed, which in turn can lead to other complications and eventually death³.

Traditional eye medicines (TEMs) are widely used in different countries for the treatment of ocular diseases. The products used vary from healer to healer and from country to country. In Nigeria, as in other developing countries, the demand for eye care is growing and needs to be supported. Some of the herbs used by

traditional healers have proven to be effective and accessible^{4,5}. The reliance on traditional eye medicines (TEMs) for care and treatment is attributed to several reasons: few trained eye doctors (ophthalmologists and optometrists), poverty and inadequate/remote health facilities for the majority of people. The disadvantage of TEMs is that the methods and products used in the treatment can lead to serious health problems, including blindness, as they are often crudely prepared. Although TEMs are widely used in many African countries, including Nigeria, there is very little documentation on plants/products used for traditional eye treatment. In addition, the right processing, formulation, dosage and efficacy of these TEMs remain very doubtful. The focus of this study is to reduce the visually impaired population through the use of Traditional Eye Medicines (TEMs). Therefore, this work aimed at evaluating the use of herbs for the treatment of ocular diseases and prevention of visual impairment.

II. Materials And Methods

The study area covers parts of the south-eastern states of Nigeria. The study area is located in the typical tropical rainforest with its great diversity of medicinal plants of different families and genera. People use various natural/traditional medicinal herbs for various ailments including ocular diseases caused by different bacteria, fungi and parasites. The people are engaged in different activities including farming, fishing, mining and several artisanal trades without personal protective equipment, which exposes them to ocular contaminants and infections. The study was conducted at the Abia State University, Uturu, where facilities for the project are available.

Swab Specimen Collection for microbiological analysis

Patients attending Abia State University, Uturu Optometry Clinic were screened for microbial infections of the eye. Patients' tears were collected for cultures using Schirmer strips. Patients were asked to answer a well-structured questionnaire to obtain information on gender, age and other data relevant to the work. Schirmer strips were used to collect samples of ocular fluid, which were inoculated onto commercially available laboratory culture media: Nutrient Agar, McConkey Agar and Blood Agar to isolate heterotrophic bacteria and coliforms and observe haemolysis of the isolates. The inoculated plates were incubated at 37°C for 24-48 hours and observed for bacterial growth. The observed microbial growth was subcultured to obtain pure isolates, which were stored in slants as stock cultures. The isolates were characterized by colonial morphology, microscopy and biochemical tests and carefully compared with the identification results as described in Cappuccino and Sherman⁶, as well as Buchanan and Doyle⁷.

Extraction of herbs crude

The medicinal plants used for the study were *Bryophyllum pinnatum*, *Occimum gratissimum* and *Aspillia africana*. These plants were obtained from the Botanical Garden of Abia State University, Uturu and used for this work. Only healthy leaves of the plants were used, which were washed in sterile distilled water and dried in the shade at room temperature. These dried leaves were separately pulverised and ground to a fine powder. 10 g of each was soaked in 50 ml of 70% ethanol for 6 hours with hourly stirring to extract the active constituents (phytochemicals). The mixture was filtered through No. 1 Watman filter paper and subjected to phytochemical analysis as indicated below:

Phytochemical analysis

The different extracts were analysed quantitatively. For the presence of alkaloids, cyanogenic glycosides, saponins, flavonoids, steroids, tannins, terpanoids and phenoids using the standard methods described by Egwaikhide *et al.*⁸.

Quantitative Analysis

A) Test for tannins: 2 ml of the extract was diluted in 10 ml of distilled water in test tubes and heated for a few minutes in a water bath. Then 1.0 ml of 1% ferric chloride (FeCl₃) was added to the mixture and observed for a dark precipitate⁸.

B) Test for saponins: Foam test: 2.0 ml of each extract was diluted in 10 ml of distilled water in test tubes and placed in a water bath for 5 minutes. The tubes were then removed, shaken vigorously and observed for visible changes. The formation of stable foam (foam or bubble) indicates the presence of saponins.

C) Test for flavonoids: To test for the presence of flavonoids in the extracts, 2.0 ml of the extracts were diluted in 10 ml of distilled water. A few pieces of aluminium metal were introduced into each tube to the level of the mixtures. Concentrated HCl was then added and allowed to stand for about 10 minutes. Each tube was examined at the end of the reaction for the formation of an orange-red colour.

D) Test for cyanogenic glycosides: 2ml of each extract was diluted in 2ml glacial acetic acid and 1-2 drops of FeCl₂ solution were added to the tubes. 2.0ml of 1% H₂SO₄ was carefully added to the mixture to form a layer or ring. A reddish-brown colour at the interface indicates the presence of cyanogenic glycosides.

E) Test for alkaloids: 2 ml of the extracts were diluted in 5 ml of 1% H₂SO₄ and heated in a water bath for 5 minutes. An equal volume of the mixture was divided into two different test tubes. 1-2 drops of Dragendorff's reagent were added to the first tube and Mayer's reagent to the other. An orange-red precipitate from one of the two tubes indicated the presence of alkaloids.

(F) Test for steroids: In this test, the Liebermann test was used⁹. Exactly 2.0 ml of acetic anhydride was added to the test tubes containing 2.0 ml of extracts in 2.0 ml of 1% H₂SO₄ and observed for a visible change. A change in the violet colour to blue or green in the tubes indicates the presence of steroids.

G) Test for terpenoids: The Salkowski test, as described by(8) Egwaikhide *et al.*⁸, was used to test for terpenoids.

Antibiotic susceptibility testing

The isolated microbial pathogens were subjected to antibacterial susceptibility testing using the Kirby-Bauer Agar diffusion technique with conventional antimicrobials for the treatment of ocular microbial diseases. The pure isolates were cultured in nutrient broth and brought to a turbidity equivalent to the 0.5 McFarland standards. Each pure isolate was inoculated onto Muller Hinton's Sensitivity Agar using the spread plate technique and allowed to stand for 30 minutes before 6mm holes were punched in the inoculated plates.

III. Results

Three hundred and seventy-seven people were examined for microbial contamination of the eye. Of these, 102 (27.05%) were male, while 275 (72.94%) were female. Of the 102 males, 17 (16.66%) had microbial contamination of the eyes and 74 (26.90%) females had microbial contamination of the eyes. The females were statistically more likely to be infected than the males ($P=0.05$). Figure 1 shows the demographic characteristics of the study population. The most infected individuals were farmers (36.58%), followed by the unemployed (29.41%), while the least infected were lecturers/teachers (10.20%), and followed by civil servants (12.28%). Statistical analysis revealed that occupational influence affected the prevalence of ocular microbial contamination ($P=0.05$).

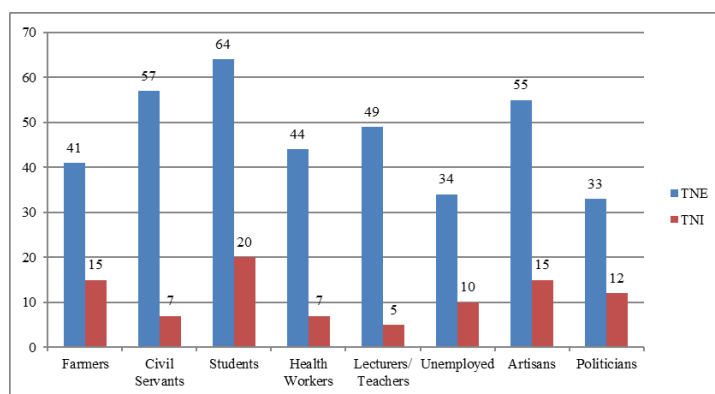


Figure 1: Occupational influence on bacterial ocular contamination

Figure 2 shows the age-related effects on the microbial contamination of the eyes of the persons examined. The most infected age group was the 61+ age group (41.44%), followed by the 31-35 age group with 32.25% and the 46-50 age group. The least infected were the 21 to 25-year-olds (8.88%) and then the 26 to 30-year-olds (15.09%). There was no clear trend in age infection ($P=0.05$). Other infection rates by age group are also shown in Figure 3.

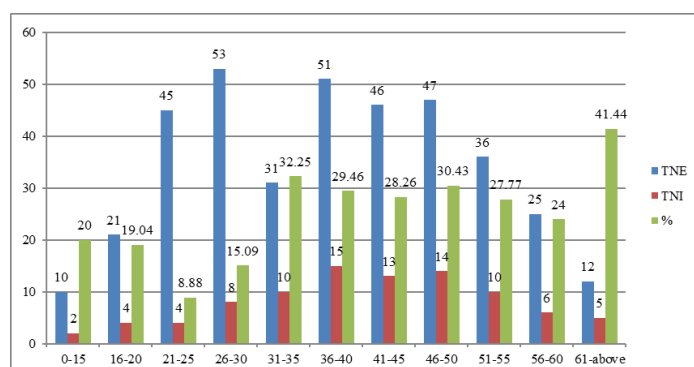


Figure 2: Age-related prevalence of ocular bacterial contaminants.

The symptoms observed in the people examined are shown in Figure 4 below. The observations showed that most of the studied subjects suffered from ocular itching (31.57%), followed by reddened eyes (27.69%), while 22.42% had discharge of various types. 24.00% had blurred vision, 20.68% had pain in the eyes and 20.00% showed no clear diagnostic signs but were microbially contaminated (Figure 4). The differences in diagnostic signs showed no significant statistical differences at a confidence level of $P=0.05$.

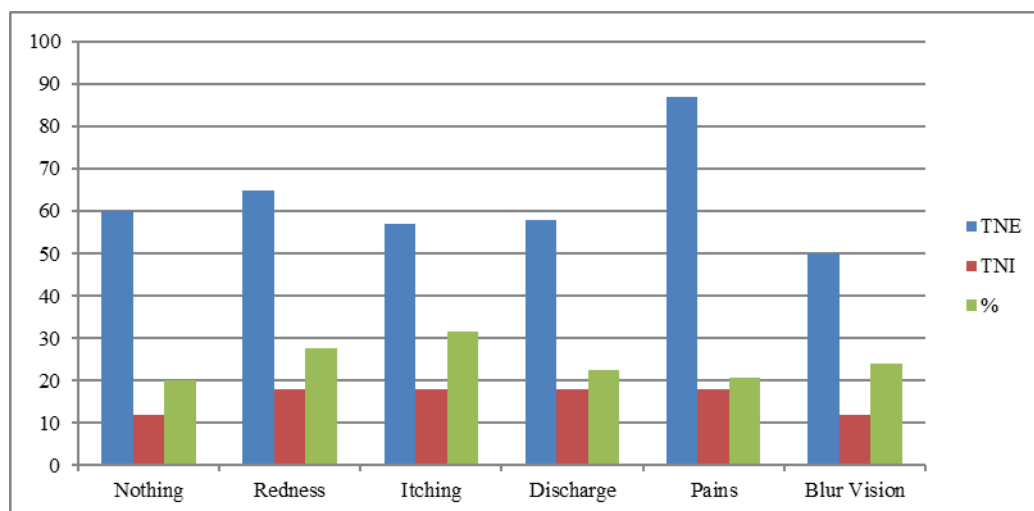


Figure 3: Various symptoms observed in the infected individuals with bacterial ocular contamination.

In this study, six bacterial species were isolated from the individuals, which were analyzed according to the main symptoms observed in the patients. Some of the patients had combined signs, especially those with discharge and redness. However, the fewest microorganisms were isolated from the patients without pronounced signs. On the other hand, the patients with ocular discharge had the highest number of microbial isolates (37), followed by the patients with ocular pain (34) and the patients without clear signs with only three microbial isolates (Table 1).

The most frequently observed bacterial species was *S. aureus*, followed by *Escherichia coli* before *Pseudomonas aeruginosa*. The least frequently reported bacterial species was *Klebsiella pneumoniae* (Table 1). The observed signs were statistically significant ($P=0.5$) in the frequency of bacterial species observed in this study.

Table 1: Bacterial species isolated from each ocular symptom observed.

Isolates	Nothing	Redness	Itching	Discharge	Pains	Blur Vision
<i>Bacillus cereus</i>	0	3(2)	3(2)	2	8(3)	3(2)
<i>Escherichia coli</i>	2	1	2(1)	5(3)	5(2)	3(2)
<i>Staphylococcus aureus</i>	1	3(1)	4	7(2)	8(3)	1
<i>Streptococcus pyogenes</i>	0	3(1)	3(1)	9(1)	8(2)	3
<i>Pseudomonas aeruginosa</i>	0	1	3(2)	6	5(3)	2
<i>Klebsiella pneumoniae</i>	0	3	3	6(3)	2(1)	5 (3)
TOTAL	3	11	14	37	34(5)	13(4)

NB: Most of the symptoms showed contamination with more than one bacterial species. Figures in bracket indicate the number of bacterial isolates observed in the particular patient

Phytochemical constituents of the plants

The observations on the different phytochemical constituents of the three plants used in this work are shown in Figure 4. There was no clear trend or gradation in the phytochemical properties. Each plant had its own constituents that differed from the others. However, the flavonoids and alkaloids had the highest values in each plant, while the steroids had the lowest values in each of the plants. The levels of tannins, saponins, phenoids and cardiac glycosides varied without any pattern. It was also found that all the phytochemicals analysed were present in all the three plants studied (Figure 4).

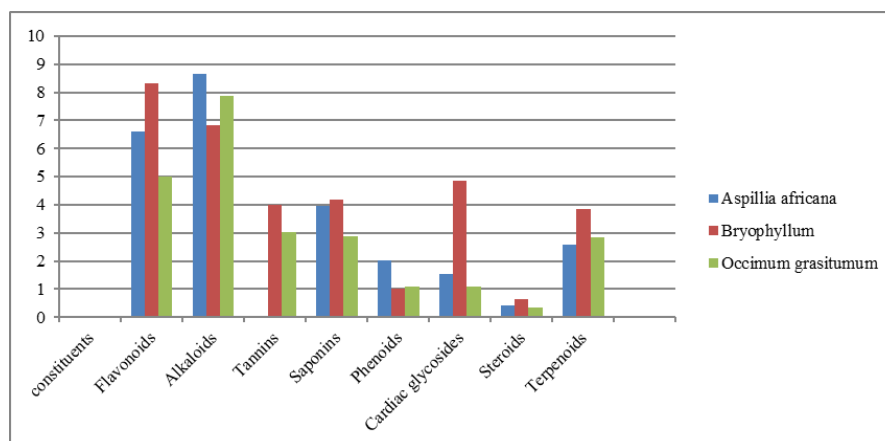


Figure 4: Quantitative phytochemical screening of *Occimum gratissimum* extracts Values are mean of three replicates.

Figures 5, 6 and 7 show the sensitivity of the six bacterial species isolated from the different samples analysed to 100%, 75% and 50% of the respective herbal extracts. For each herbal extract, 100% had the highest inhibitory effect on each of the isolates. This was followed by 75%, while 50% of each herbal extract had the least inhibitory effect on each of the isolates. This shows a clear trend that high concentrations have a higher inhibitory effect on the isolates, while lower concentrations have a low inhibitory effect ($P=0.5$). The 100% concentration of each herbal extract had an inhibitory effect that was close to the control values with cefuraxin, but not statistically significant ($p=0.5$). However, the extracts of *Bryophyllum pinnatum* had the strongest inhibitory effect, followed by *Occimum gratissimum*, while the extracts of *Aspilia africana* showed the least effect (Figures 5, 6 and 7).

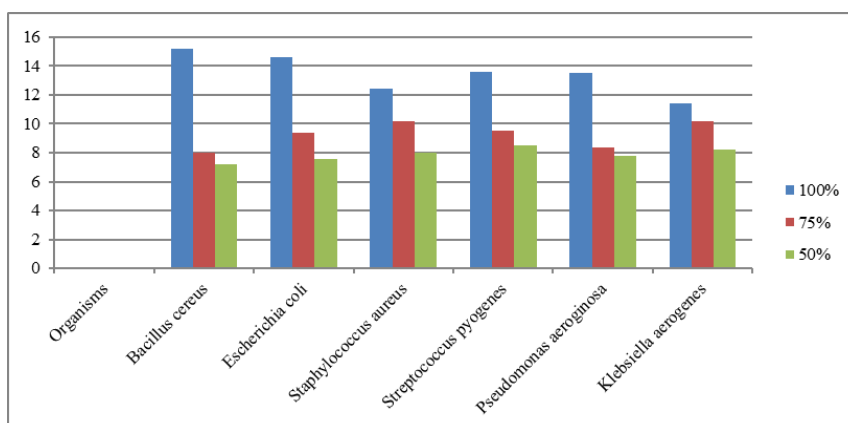


Figure 5: Antimicrobial activity of *Aspilia africana* extracts against bacterial isolates from the eyes (mm)

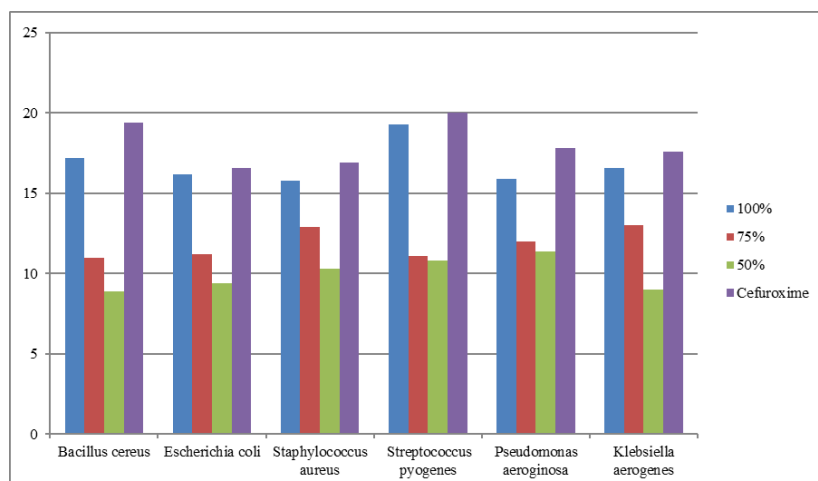


Figure 6: Antimicrobial activity of *Bryophyllum pinnatum* extracts against bacterial isolates from the eyes (mm)

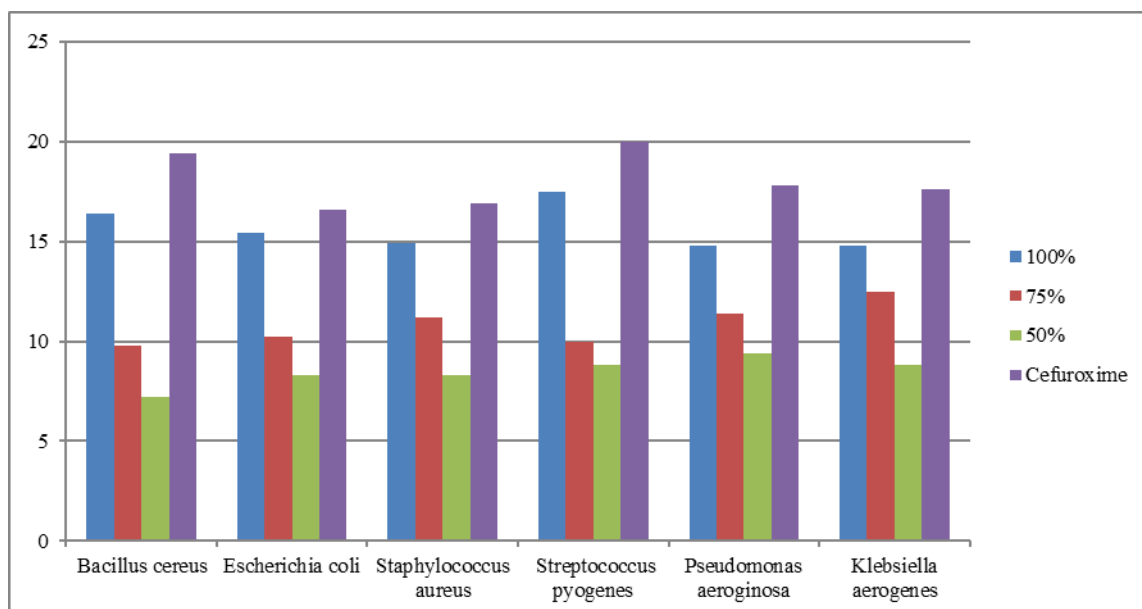


Figure 7: Antimicrobial activity of *Occimum gratissimum* extracts against bacterial isolates from the eyes ulcer (mm)

The observations in Tables 2, 3 and 4 shows the response to experimental in vivo infection of albino rats with the bacterial isolates treated with the 100% concentrations of each herbal extract. The extracts of *Aspillia Africana* began to recover on day 6 of experimental infection with *Escherichia coli* and *Streptococcus pyogenes*, while *Klebsiella pneumoniae* showed no recovery until day 8.

Bryophyllum pinnatum showed recovery of four bacterial isolates except *Staphylococcus aureus* and *Klebsiella pneumoniae* by day 6 (Table 3). The observations made with *Occimum gratissimum* were similar to those of *Aspillia africana* (Tables 2 and 4). The observations showed that the herbal extracts had differences in bacterial isolates ($P=0.05$).

Table 2: In vivo antibacterial activities of *Aspillia africana* on the experimental bacterial infections (days)

Organisms	0	2	4	6	8	10	12	14	16	18
<i>Bacillus cereus</i>	-	-	-	-	+	+	+	+	+	+
<i>Escherichia coli</i>	-	-	-	+	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	-	-	+	+	+	+	+	+
<i>Streptococcus pyogenes</i>	-	-	-	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	-	-	+	+	+	+	+	+
<i>Klebsiella aerogenes</i>	-	-	-	-	-	+	+	+	+	+

Table 3: In vivo antibacterial activities of *Bryophyllum pinnatum* on the experimental bacterial infections (days)

Organisms	0	2	4	6	8	10	12	14	16	18
<i>Bacillus cereus</i>	-	-	+	+	+	+	+	+	+	+
<i>Escherichia coli</i>	-	-	+	+	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	-	+	+	+	+	+	+	+
<i>Streptococcus pyogenes</i>	-	-	+	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	+	+	+	+	+	+	+	+
<i>Klebsiella aerogenes</i>	-	-	-	-	+	+	+	+	+	+

Table 4: In vivo antibacterial activities of *Aspillia africana* on the experimental bacterial infections (days)

Organisms	0	2	4	6	8	10	12	14	16	18
<i>Bacillus cereus</i>	-	-	-	+	+	+	+	+	+	+
<i>Escherichia coli</i>	-	-	-	+	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	-	-	+	+	+	+	+	+

<i>Streptococcus pyogenes</i>	-	-	-	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	-	+	+	+	+	+	+	+
<i>Klebsiella aerogenes</i>	-	-	-	-	-	+	+	+	+	+

IV. Discussion

The treatment of eye diseases with drugs that have no side effects remains a problem for the medical system. The medical system continues to struggle with treating eye diseases using chemical treatments that have adverse effects¹⁰. Medicinal plants have the ability to overcome the limitations associated with conventional drugs. Due to their efficacy, low side effects and cost-effectiveness, several attempts have been made to discover new herbal medicines from a variety of sources¹⁰. This current study investigated the effect of *Bryophyllum pinnatum*, *Occimum gratissimum* and *Aspillia africana* in the treatment of bacterial ocular infections. The findings revealed that out of 102 males, 17 (16.66%) had microbial contamination of the eyes, while 74 (26.90%) females showed similar contamination, suggesting that females are more susceptible to bacterial ocular infections. The highest incidence of infection was observed in farmers (36.58%) followed by unemployed (29.41%). The most affected age group was the over 61-year-olds (41.44%), closely followed by the 31-35 age group (32.25%). Those studied suffered from itchy eyes (31.57%), with reddened eyes (27.69%) being the second most common symptom, while 22.42% reported various types of discharge. The most commonly identified bacterial species was *S. aureus*, followed by *Escherichia coli* and then *Pseudomonas aeruginosa*. Phytochemical analysis showed that flavonoids and alkaloids were present in the highest concentrations in each plant. The presence of growth inhibition zones upon application of the extract after a certain incubation period indicates the degree of antimicrobial activity of the extract against the tested organisms. In this study, the extracts of *Bryophyllum pinnatum* showed the strongest inhibitory effect, followed by *Occimum gratissimum*, while the extracts of *Aspillia africana* showed the least effect. The differences in the sensitivity of the microorganisms to the different leaf extracts could be due to the chemical composition of the extract, certain specific internal factors of each test microorganisms and the environment¹¹. This finding confirmed the antimicrobial activity of the constituents of the extract and their potency in treating various ailments caused by different bacterial pathogens, protozoa and fungi^{4,12}.

V. Conclusion

The findings of this study provide further evidence for the use of extracts of *Bryophyllum pinnatum*, *Occimum gratissimum* and *Aspillia africana* in the treatment of bacterial-related ocular diseases. The extracts of *Bryophyllum pinnatum* showed the strongest inhibitory effect, followed by *Occimum gratissimum*, while the extracts of *Aspillia africana* showed the least effect. The different susceptibility of the microorganisms to plant extracts could be due to the chemical composition of the extracts. This study suggests that these plants have the potential to be used in the treatment of ocular diseases caused by bacterial infections. However, further studies are needed to determine the effective doses for the treatment of bacterial eye infections without side effects.

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