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Healing Effects Of Cocos Nucifera, Dacryodes Edulis And Garcinia Kola Extracts In Induced Corneal Ulcers In Rabbits

A.C. Ezeigbo, A. U. Omaka, C.I. Nosiri, C.O. Timothy, A.O. Ebere, J. E. Ekeleme, C.A. Anonaba, I.C. Benjamin

(Department Of Optometry, Faculty Of Health Sciences, Abia State University, Uturu, Abia State, Nigeria)
(Department Of Pharmacy, Faculty Of Pharmaceutical Sciences, Abia State University,Uturu, Abia State, Nigeria)
(Department Of Microbiology, Faculty Of Biological Sciences, Rhema University, Aba, Abia State, Nigeria)

Abstract
Background: *Corneal ulcer refers to a break in the cornea's surface epithelium and associated with necrosis of the surrounding tissue. It is a major cause of ocular morbidity and blindness worldwide and presents a significant therapeutic challenge due to growing antimicrobial resistance as well as restricted access to conventional treatments in developing countries. This drives the study of alternative therapies, such as medicinal plants with established ethnomedicinal effects. The ethanolic extracts of Cocos nucifera, Dacryodes edulis, and Garcinia kola were investigated in this study for their healing properties in induced corneal ulcers and an alternative to orthodox medications.*
Materials and Methods*: The prevalence and microbial aetiology of corneal ulcers in 381 patients attending the Optometry clinic in Abia State University was determined through a clinical survey. The phytochemical contents of the ethanolic extracts of Garcinia kola*, *Cocos nucifera, and Dacryodes edulis were quantitatively evaluated. The antimicrobial efficacy of the ethanol extracts against clinical isolates was tested in vitro using conventional culture techniques and compared with conventional antimicrobials. The healing effects of different concentrations of the plants extracts was further assessed on experimentally induced corneal ulcers in rabbits over 14 days using a hand-held slit lamp and pupillary distance rule.*
Results: *The prevalence of microbial corneal ulcers was 40.94%, with higher rates among younger age groups and farmers. The study also revealed a higher prevalence rates in females (60.86%) than in males (53.33%). Aspergillus flavus (63.46%) and* Staphylococcus aureus *(62.82%) were the predominant micobial isolates of fungi and bacteria respectively. All three plant extracts were rich in flavonoids, alkaloids, cardiac glycosides, and saponins. While saponins had higher concentrations in Dacryodes edulis*, the rest were revealed in greater concentrations in Garcinia kola. *The 100% plant extract concentration exhibited potent antimicrobial activity, producing zones of inhibition statistically similar to standard controls (Amikacin, Amphotericin B), and promoted healing in rabbit models. In vivo, extracts of Garcinia kola and Cocos nucifera initiated healing by day 3-4 for most pathogens, while Dacryodes edulis showed effects by day 4.*
Conclusion: *Ethanolic extracts of Garcinia kola*, *Cocos nucifera*, *and Dacryodes edulis show significant antimicrobial and wound-healing properties. These locally available plants represent promising, effective and affordable alternatives for the management of corneal ulcers, mostly in settings confronted with antibiotic resistance.*
Keywords: *Corneal ulcer, Antimicrobial resistance, Medicinal plants, Garcinia kola, Phytochemicals, Alternative therapy.*

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I. Introduction

Corneal ulcers represent a significant ocular condition characterized by a breach in the corneal epithelium resulting in underlying tissue necrosis and inflammatory infiltration[1,2]. As the eye's outermost transparent layer, the cornea is persistently vulnerable to injury from environmental exposures, leading to abrasions, lacerations, and ulcerations compromising vision[3]. These ulcers present as gray-white opacities on the typically clear corneal surface and constitute both a medical emergency and a predominant cause of preventable blindness, particularly in developing nations with limited access to eye care delivery[4]

The etiology of corneal ulcers is multifaceted, encompassing infectious agents including bacteria, fungi, parasites and viruses[5], as well as non-infectious causes such as trauma, autoimmune conditions, chemical injuries, entropion, trichiasis, blepharitis, lagophthalmos, cuts and abrasions[6,7]. Clinical manifestations typically include

severe pain, photophobia, blepharospasm, redness, and blurred vision, with potential complications ranging from corneal opacity and perforation to permanent visual impairment[4,8]. Pathogenically, bacterial adherence to corneal epithelial cells initiates a destructive process mediated by microbial proteinases that degrade the extracellular matrix, facilitating stromal invasion and potentially leading to perforation[2].

Conventional management of corneal ulcers relies heavily on severity as well as antimicrobial therapies, with treatment success contingent upon accurate microbiological diagnosis and susceptibility testing[9,10,11] However, the escalating global crisis of antimicrobial resistance, coupled with challenges in diagnostics and rising treatment costs, has substantially undermined the efficacy of standard antibiotic regimens[12]. This therapeutic shortfall is especially critical in resource-limited settings, where corneal ulcers remain prevalent and often progress to blindness due to delayed or inadequate treatment[13].

In light of these challenges, medicinal plants have emerged as promising sources of alternative therapeutics due to their rich collection of bioactive compounds with demonstrated antimicrobial and anti-inflammatory properties[14]. Phytochemicals such as flavonoids, alkaloids, tannins, and saponins exhibit broad-spectrum activity against pathogens and can modulate host inflammatory responses, thereby addressing both infectious and pathological components of corneal ulceration[14]. Among Nigerian flora, *Garcinia kola* (bitter kola), *Cocos nucifera* (coconut), and *Dacryodes edulis* (African pear) have been reported to be ethnomedicinally relevant. *Garcinia kola*, traditionally termed a "miracle plant," is renowned for its diverse pharmacological applications, including antimicrobial and anti-inflammatory effects[15,16,17] and it is readily served to visitors, especially among the Igbo tribe in Eastern Nigeria, as a sign of peace and acceptance of visitors[15]. *Cocos nucifera*, a member of the palm family Arecaceae and abundant in tropical regions of the world[18] possesses documented antioxidant, antimicrobial, and anti-inflammatory activities[19], while *Dacryodes edulis* has been investigated for its phytochemical properties, therapeutic potential as well as spiritual and economic values[20,21,22,23].

Despite their traditional use, robust scientific evaluation of these plants for treating ocular infections remains limited. Given the urgent need for effective, accessible, and affordable alternatives to conventional antibiotics, this study aims to investigate the efficacy of ethanolic extracts from *Garcinia kola, Cocos nucifera,* and *Dacryodes edulis* in promoting the healing of experimentally induced corneal ulcers. The findings may contribute to the development of phytotherapeutic strategies to mitigate corneal blindness, particularly in underserved populations where antimicrobial resistance and limited healthcare access pose dual challenges.

II. Materials And Methods

Study Design: The research was a prospective observational study which used the convenient sampling technique.

Study Location: The study location was Abia State University, Uturu, South East Nigeria. The University's Optometry clinic served as the reference clinic.

Study Duration: May 2021 to November 2021.

Sample size: Three hundred and eighty-one human subjects (381) subjects and 45 adult rabbits.

Sample size calculation: The study was based on convenience sampling, so no sample size calculation was done.

Subjects and selection method: The outpatients who assessed the Optometry Clinic facilities of the University within the period of recruitment were enlisted for the study, especially those suspected to have corneal ulcer.

Inclusion criteria:
1. Patients who presented at the Optometry Clinic with symptoms and signs highly suggestive of a corneal ulcer
2. Those who were willingly to participate.

Exclusion criteria:
1. Those who were unable to consent to the study.
2. Patients with perforating injuries or extensive ocular trauma requiring immediate surgical intervention.
3. Patients who had already commenced treatment with topical or systemic antibiotics or antifungal medications prior to sample collection.
4. Those with underlying systemic immunocompromising conditions.

Plant Collection and Identification

Fresh fruit samples of *Cocos nucifera* (Coconut), *Dacryodilis edulis* (African pea) and *Garcinia kola* (Bitter kola) seeds were collected from Uturu, Abia State, South East, Nigeria. They were properly identified and authenticated at the Department of Plant Science and Biotechnology of the University before use.

Phytochemical Analysis

A quantitative analysis of the phytoconstituents of the plant materials was conducted using standard protocols outlined by the Association of Official Analytical Chemists (AOAC) standard methods[24]. The screened bioactive compounds included alkaloids, flavonoids, tannins, saponins, phenols, cardiac glycosides, steroids, and terpenoids.

Data Collection

Demographic and clinical data from patients with corneal ulcers were collected using a well-structured questionnaire. Informed consent was obtained from all participants after a detailed explanation of the study's purpose and data usage.

Extract Preparation

The collected plant materials were washed with tap water, air-dried at room temperature, and pulverized into a fine powder using a laboratory blender. The crude ethanolic extract was obtained through maceration using a 1:3 (w/v) plant-to-ethanol ratio for 8 hours with intermittent agitation. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator to remove the ethanol. The resulting crude extract was stored in sealed glass bottles at 6°C till required for use.

Isolation of Microbial Species

Ocular fluid samples from patients were collected using Schirmer's strips. Samples were streaked onto Nutrient Agar and MacConkey Agar for bacterial isolation and Sabouraud Dextrose Agar (SDA) for fungal isolation. Inoculated Petri dishes were incubated aerobically at 37°C for 24–48 hours for bacterial cultures and at room temperature for 2–4 days fungal cultures. Pure isolates were obtained through sub-culturing and identified based on visual characterization, microscopic examination, after staining, and biochemical profiles[25]. Fungal identification and characterization were further based on colonial features and microscopic characterization, especially using the pigmentation and fruiting bodies features[26].

Animal Studies

Forty-five adult rabbits were acquired and housed separately in stainless steel cages with free access to dry pelletized feeds (Vital Feed ®, Nigeria) and water *ad libitum*. The animals were acclimatized for 14 days under ambient laboratory conditions and 12-hr light/dark conditions, before the commencement of the study.

Induction of Corneal Ulcers

After topical anesthesia with Proparacaine hydrochloride, corneal ulcer was induced in the left eye of each rabbit by applying a sterile Schirmer's strip saturated with 1N sodium hydroxide (NaOH) for 10 seconds. The eye was immediately irrigated thrice with normal saline to neutralize the alkali. Ulcer induction was confirmed using fluorescein staining, examined with a handheld biomicroscope and a pupillary distance rule for shape and size[27].

Antimicrobial Susceptibility Testing

The Kirby-Bauer disc diffusion technique was employed for standard antibiotic testing. Bacterial and fungal isolates were inoculated onto Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA), respectively. The antibiotic-impregnated discs were aseptically placed on the inoculated agar surfaces to have full contact in a well-spaced manner. Plates were incubated at 37°C for 24–48 hours for bacteria and at room temperature for 2–3 days for fungi. Diameter zones of inhibition were measured in mm using transparent rule.

Each plant extract was diluted to 75%, 50%, and 25% concentrations, with the undiluted extract serving as the 100% concentration, with Agar well diffusion approach (Kirby Bauer techniques). Each microbial isolate was inoculated on MHA (bacteria) and SDA (fungi) using the spread plate technique. 6mm diameter wells were made in the Agar using sterile cork-borer in well-spaced manner and filled with the different extract concentrations. Plates were left for 20–30 minutes to allow for diffusion into the Agar and then incubated anerobically, 24-48 hours for bacteria and 2-3 days for fungi. The resulting diameter zones of inhibition were measured in mm.

Evaluation of Plant Extract Efficacy (In Vivo)

The 45 rabbits were randomly divided into three treatment groups (A, B, C) of fifteen each. The induced ulcers were inoculated with microbial isolates. The initial diameter of each corneal ulcer was determined before treatment commencement. Each group was treated topically with two drops (≈0.12 mL) of a 100% reconstituted (ratio of one gram of the extract to 2 ml of sterile distilled water) extracts of *Cocos nucifera, Dacryodes edulis,* or- *Garcinia kola* every 6 hours for 14 days. This was because the 100% concentration gave the best results on the isolates in vitro.

Statistical Analysis

The data were analyzed using SPSS Version 27 and expressed as mean ± standard error. Mean differences were tested using student's T-test, while differences between the groups were separated by a one-/two-way analysis of variance (ANOVA). Values were considered significant at $P \leq 0.05$.

III. Results

Figure 1 shows the phytochemical constituents of the three plants extracts. These include flavonoids, alkaloids, tanins saponins and phenoids. Others were cardiac glycosides, steroids and terpenoids. The Flavonoids (1.08±0.02 to 5.44+0.02), and Cardiac glycosides (1.40±0.02 to 4.87±0.02) have their highest values in the *Garcinia kola*, while saponins (1.20±0.02 to 2.46±0.02) and steroids (0.20±0.02 to 1.00±0.02) highest values were in the *Dacryodes edulis*.

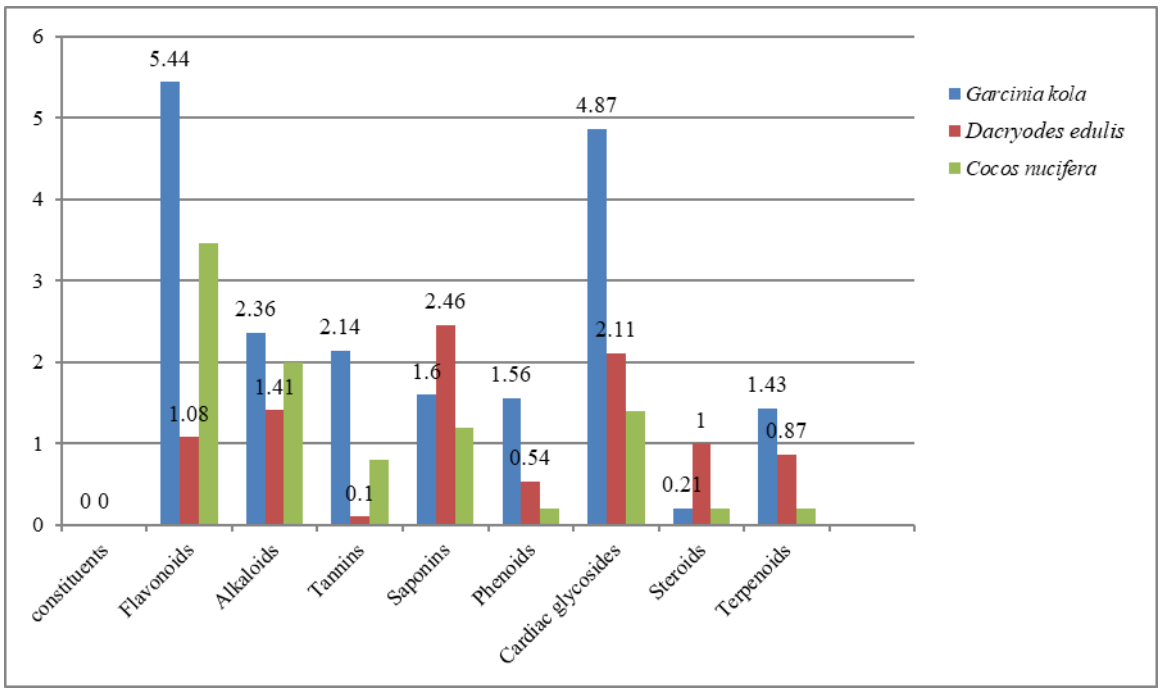

Figure 1: Phytochemical constituents of the three plants used in the study.

Table 1 shows the age and gender-related influence on the prevalence of cornea ulcer trauma in the study. Among the 381 people, 205 were males with 79 (38.53%) having corneal ulcer and 77 (43.75%) had corneal ulcer among the 176 females. The highest prevalence was 54.16% (36-45years). Females had a higher prevalence rate of 60.86% (36-45years) than the males 53.33% (0-15 years). The least was seen in the 66 years and above group.

Table 1: Age and gender related influence on prevalence of Corneal ulcer on target population

Age (years)	MS	MI	FS	FI	TNS	TNI
0-15	15	8(53.33)	10	5(50.00)	25	13(52.00)
16 - 25	20	10(50.00)	25	14(56.00)	45	24(53.33)
26 - 35	30	11(36.66)	35	16(45.71)	65	27(41.53)
36 - 45	25	12(48.00)	23	14(60.86)	48	26(54.16)
46- 55	30	13(43.33)	21	10(47.61)	51	23(45.09)
56 -65	40	15(37.50)	32	10(31.25)	72	25(34.72)
66-Above	45	10(22.22)	30	8(26.66)	75	18(24.00)
Total	205	79(38.53)	176	77(43.75)	381	156(40.94)

Keys: MS: Males Screened MI: Males Infected FS: Females Screened FI: Females Infected
TNS: Total Number Screened TNI: Total Number Infected

Table 2 presents the occupation-related influence on the prevalence of corneal ulcer in the study.

Farmers had the highest prevalence in both males (59.52%) and females (61.54%), while the least prevalence were seen among the students (18.51%) and teachers/lecturers (20.00%) in males and females respectively. Statistical analysis showed that there was significant occupational influence on the distribution of corneal ulcer among the target population (P=0.05).

Table 2: Occupation-related influence on prevalence of Corneal ulcer on target population

Occupation	MS	MI	FS	FI	TNS	TNI
Farmers	42	25(59.52)	26	16(61.54)	58	41(60.29)
Civil Servants	27	9(33.33)	18	7(38.88)	45	16(31.11)
Students	27	5(18.51)	49	24(48.97)	76	29(38.15)
Health workers	11	5(45.45)	16	6(37.50)	27	11(33.33)
Teachers/lecturers	15	4(26.66)	20	4(20.00)	35	8(37.14)
Artisans	30	11(36.66)	19	11(57.89)	49	22(51.02)
Politicians	24	10(41.66)	11	3(27.27)	35	13(37.14)
Unemployed	29	10(34.48)	17	6(35.29)	46	16(34.78)
Total	205	79(38.53)	176	77(43.75)	381	156(40.94)

Keys: MS: Males Screened MI: Males Infected FS: Females Screened FI: Females Infected TNS: Total Number Screened TNI: Total Number Infected

Table 3 shows the microbial species isolated from corneal ulcer patients. The laboratory analysis of the ocular fluid showed the presence of seven (7) bacterial species and six fungal species. *Staphylococcus aureus* (62.82%) and *Aspergillus flavus* (63.46%), had the highest occurrence.

Table 3: Microbial species isolated from corneal ulcer patients population

Organism	TCP	NMI	%
Bacteria			
Staphylococcus aureus	156	98	62.82
Staphylococcus viridans	156	56	35.89
Streptococcus pyogenes	156	57	36.53
Pseudomonas aeruginosa	156	77	49.35
Pseudomonas aeruginosa	156	45	28.84
Proteus mirabilis	156	48	30.76
Klebsiella pneumoniae	156	69	44.23
Fungi			
Candida albicans	156	94	60.25
Fusarium solani	156	76	48.71
Aspergillus fumigatus	156	92	58.97
Aspergillus flavus	156	99	63.46
Aureobasidium speces	156	66	42.30
***Alternaria* species**	156	57	36.53

Key: TCP: Total Corneal ulcer Patients NMI: Number of patients with Microbes Isolated %: Percentages

Figure 2, shows antibacterial susceptibility profile of isolates. All isolates of *S. aureus, S. viridans, S.pyogenes* and *E.coli* were susceptible to Amikacin.

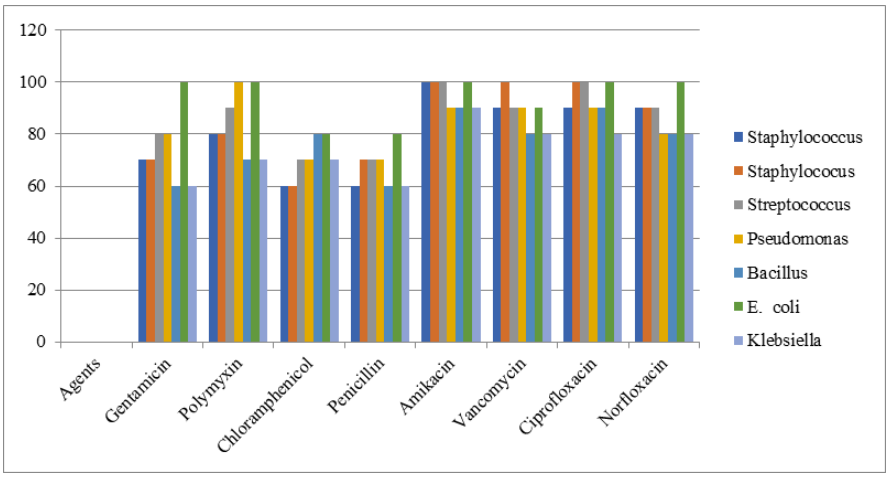

Figure 2: The susceptibility percentage of the bacterial isolates to antibiotics tested

Figure 3 shows the anti-fungal susceptibility test. Of the six (6) fungal species, *Candida albicans, Fusarium solani,* and *Aureobasidium* species showed 100% susceptibility to Amphotericin B.

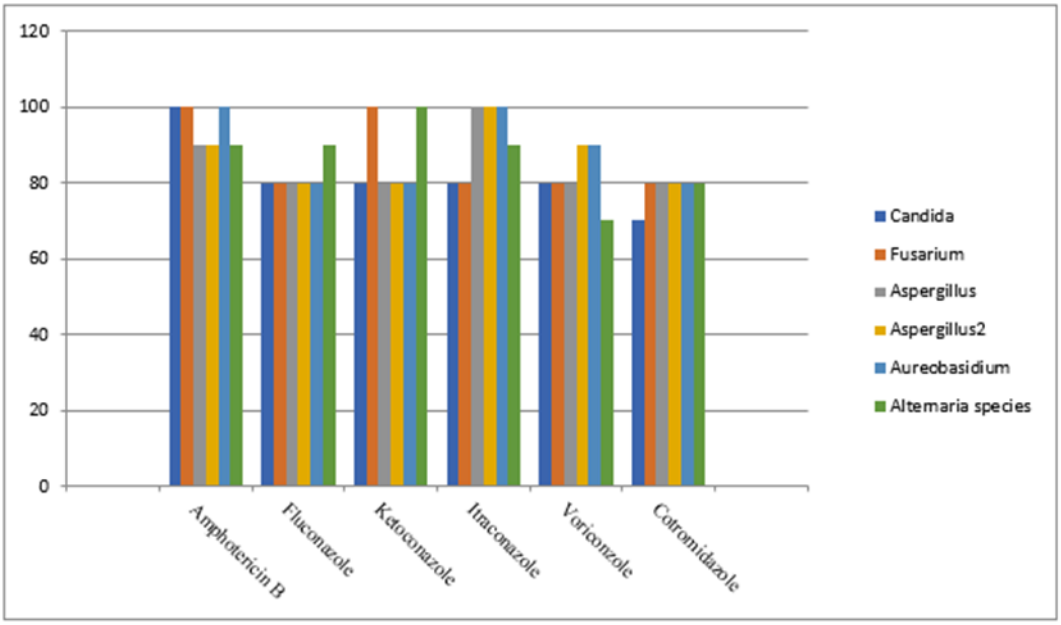

Figure 3: The susceptibility percentage of the fungal isolates to anti-fungals tested

Tables 4, 5 and 6 show the antimicrobial activity of ethanol extracts *Garcinia kola* seed, *Cocos nucifera* nut and *Dacroydes edulis* nut respectively against microbial isolates from corneal ulcer**.** The assessment of the antimicrobial activities of the plant extracts showed that the bacterial and fungal isolates were resistant to 25 and 50 per cent concentrations of the plant extracts (Tables 4-6). The isolates were all susceptible to the 100 per cent concentration of the extracts and showed diameter zones of inhibition statistically similar to the controls used (Amikacin for bacteria and Amphotericin B for fungi) (P=0.05; Tables 4 – 6).

Table 4: Antimicrobial activity of *Garcinia kola* seed ethanol extracts against microbial isolates from corneal ulcer

Organisms	25% Extract	50% Extract	75% Extract	100% Extract	Control (Amikacin)	Control (Itraconazole)
Bacteria						ND
S. aureus	0.4±0.0	7.0±0.2	12.0±0.2	14.8±0.2	17.4±0.2	
S. viridans	3.4±0.0	9.0±0.3	13.0±0.2	15.0±0.2	19.2±0.2	ND
Streptococcus pyogenes	2.6±0.0	8.4±0.2	13.0±0.2	15.0±0.2	18.6±0.2	ND
Pseudomonas aeruginosa	0.0±0.0	8.2±0.3	1230±0.2	15.6±0.3	18.9±0.3	ND
Bacillus cereus	0.0±0.0	8.5±0.3	12.0±0.0	15.5±0.2	18.0±0.1	ND
E.coli	2.0±0.0	9.2±0.1	14.6±0.3	16.3±0.2	20.2±0.2	
Proteus mirabilis	2.0±0.0	8.5±0.2	13.5±0.3	17.4±0.2	18.8±0.2	
Klebsiella pneumonias	0.0±0.0	8.6±0.3	11.1±0.2	17.5±0.2	17.3±0.2	ND
Fungi					Amphotericin B	
Candida albicans	3.0±0.0	7.7±0.2	13.1±0.2	16.4±0.0	20.1±0.2	20.1±0.2
Fusarium solani	4.5±0.0	7.6±0.2	14.1±0.3	17.2±0.2	19.4±0.2	21.1±1.0
Aspergillus fumigatus.	2.5±0.0	7.6±0.2	15.0±0.2	18.4±0.3	20.5±0.2	24.1±0.1
Aspergillus flavus.	2.8±0.0.	7.5±0.2	14.2±0.2	17.9±0.2	20.5±0.2	22.0±0.5
Aureobasidium species	3.3±0.0.	7.6±0.2	15.4±0.2	19.6±0.2	22.6±0.2	
Alternaria species	2.0±0.0	7.9±0.2	16.3±0.2	18.4±0.2	23.1±0.2	

Table 5: Antimicrobial activity of *Cocos nucifera* nut ethanol extract against bacterial isolates from corneal ulcer

Organisms	25% Extract	50% Extract	75% Extract	100% Extract	Control (Amikacin)	Control (Itraconazole)
Bacteria						ND
S. aureus	0.4±0.0	7.4±0.2	10.0±0.2	12.9±0.2	17.4±0.2	
S. viridans	3.0±0.0	8.0±0.2	11.5±0.2	13.6±0.2	19.2±0.2	ND
Streptococcus pyogenes	2.0±0.0	7.8±0.2	11.3±0.2	14.6±0.2	18.6±0.2	ND

Pseudomonas aeruginosa	0.0±0.0	6.8±0.3	12.0±0.2	15.0±0.0	18.9±0.3	ND
Bacillus cereus	1.0±0.0	8.0±0.3	10.4±0.0	13.6±0.2	18.0±0.1	ND
E.coli	2.0±0.0	8.1±0.2	12.3±0.3	13.3±0.2	20.2±0.2	
Proteus mirabilis	2.4±0.0	7.3±0.2	10.4±0.2	15.0±0.2	18.8±0.2	
Klebsiella pneumoniae	0.5±0.0	8.0±0.2	11.5±0.2	14.0±0.2	17.3±0.2	ND
Fungi					Amphotericin B	
Candida albicans	3.0±0.0	8.1±0.2	13.4±0.2	15.0±0.0	20.1±0.2	20.1±0.2
Fusarium solani	4.5±0.0	7.9±0.2	12.0±0.3	14.2±0.2	19.4±0.2	21.1±1.0
Aspergillus fumigatus.	2.0±0.0	6.3±0.2	12.2±0.2	14.0±0.3	20.5±0.2	24.1±0.1
Aspergillus flavus.	2.4±0.0.	6.4±0.2	12.1±0.2	14.0±0.2	20.5±0.2	22.0±0.5
Aureobasidium species	3.8±0.0.	8.2±0.2	12.3±0.2	14.0±0.2	22.6±0.2	
Alternaria species	2.3±0.0	6.8±0.2	12.3±0.2	15.1±0.2	23.1±0.2	

Table 6: Antimicrobial activity of *Dacroydes edulis* nut ethanol extract against bacterial isolates from corneal ulcer

Organisms	25% Extract	50% Extract	75% Extract	100% Extract	Control (Amikacin)	Control (Itraconazole)
Bacterial species						ND
S. aureus	0.0±0.0	7.0±0.2	9.1±0.2	13.2±0.2	17.4±0.2	
S. viridans	2.0±0.0	7.0±0.2	11.0±0.2	13.3±0.2	19.2±0.2	ND
Streptococcus pyogenes	1.0±0.0	7.1±0.2	9.10±0.2	14.0±0.2	18.6±0.2	ND
Pseudomonas aeruginosa	0.0±0.0	4.3±0.3	9.1±0.2	13.2±0.0	18.9±0.3	ND
Bacillus cereus	0.0±0.0	5.3±0.3	9.2±0.0	13.6±0.2	18.0±0.1	ND
E.coli	2.0±0.0	7.4±0.2	10.6±0.3	13.1±0.2	20.2±0.2	
Proteus mirabilis	1.1±0.0	7.3±0.2	10.4±0.2	13.2±0.2	18.8±0.2	
Klebsiella pneumoniae	0.0±0.0	6.3±0.2	9.5±0.2	12.3±0.2	17.3±0.2	ND
Fungal species					Amphotericin B	
Candida albicans	2.3±0.0	8.0±0.2	10.4±0.2	14.1±0.0	20.1±0.2	20.1±0.2
Fusarium solani	2.5±0.0	7.0±0.2	10.0±0.3	16.1±0.2	19.4±0.2	21.1±1.0
Aspergillus fumigatus.	2.4±0.0	5.4±0.2	11.1±0.2	17.0±0.2	20.5±0.2	24.1±0.1
Aspergillus flavus.	2.0±0.0.	5.8±0.2	11.2±0.2	15.4±0.2	20.5±0.2	22.0±0.5
Aureobasidium species	2.3±0.0.	5.6±0.2	11.4±0.2	16.8±0.2	22.6±0.2	
Alternaria species	2.0±0.0	6.0±0.2	12.0±0.2	12.9±0.2	23.1±0.2	

Tables 7 to 9 show the healing activities of the three plant extracts – *Garcinia kola, Dracryodes edulis* and *Cocos nucifera* on the experimentally induced corneal ulcer. Observations in Tables 7 and 8 showed that the first two days of challenging the isolates in the corneal ulcer of the rabbits did not yield any healing effects in both the bacterial and fungal isolates**.** However, all the corneal ulcer inoculated with the fungal isolates and some of the bacterial isolates showed healing signs by the fourth day (Table 9). By the fifth day, all isolates showed healing signs (Table 9)

Table 7: Period of healing the experimental corneal ulcer treating with ethanol *Garcinia kola* extract (days).

Organisms	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Bacterial species															
S. aureus	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
S. viridans	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
S. pyogenes	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
P. aeruginosa	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Bacillus cereus	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
E. coli	-	-	+	+	+	+	+	+	+	+	+	+	+	+	
Proteus mirabilis	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Fungal species															
Candida albicans	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Fusarium solani	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
A.fumigatus	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
A. flavus	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Aureobasidium	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Alternaria	-	-	+		+	+	+	+	+	+	+	+	+	+	+

Table 8: Period of healing the experimental corneal ulcer treating with ethanol *Cocos nucifera* nut extract (days).

Organisms	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Bacterial species															
S. aureus	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
S. viridans	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
S. pyogenes	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
P. aeruginosa	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Bacillus cereus	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
E. coli	-	-	+	+	+	+	+	+	+	+	+	+	+	+	
Proteus mirabilis	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Fungal species															
Candida albicans	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Fusarium solani	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
A.fumigatus	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
A. flavus	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Aureobasidium	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Alternaria	-	-	+		+	+	+	+	+	+	+	+	+	+	+

Table 9: Period of healing the experimental corneal ulcer treating with ethanol *Dacryodes edulis* extract (days).

Organisms	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Bacterial species															
S. aureus	-	-		+	+	+	+	+	+	+	+	+	+	+	+
S. viridans	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
S. pyogenes	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
P. aeruginosa	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Bacillus cereus	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
E. coli	-	-	-	+	+	+	+	+	+	+	+	+	+	+	
Proteus mirabilis	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Fungal species															
Candida albicans	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Fusarium solani	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
A.fumigatus	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
A. flavus	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Aureobasidium	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Alternaria	-	-	+		+	+	+	+	+	+	+	+	+	+	+

IV. Discussion

The use of plant extracts as an alternative to conventional medicine has become an issue of increasing interest due to the disturbing issues surrounding conventional drugs of which antimicrobial resistance is the most. The Phytochemical components showed that flavonoids, phenolics, alkaloids and cardiac glycosides, were highest in *Garcinia kola,* these findings were agreement with studies which also noted the presence of active ingredients in their works on *Garcinia kola*[28,29]. Saponins, tannins and phenols were found most in *Dacryodes edulis.* This was also consistent with the findings of Alviano and Alviano[30] as well as Okwu and Nnamdi[31] who worked on the Evaluation of The Chemical Composition of *D. edulis* and *R. hookeri mann* and Wendl exudates used in herbal medicine in south eastern Nigeria. *Cocos nucifera* however showed low levels of tannins, phenols and very minute amounts of alkaloids and terpenoids. This presented from our findings that *Garcina kola* had the richest source of bioactive groups followed by *D. edulis* and then *C. nucifera.* Some other scientists however showed varying results on the quantitative analysis of the phytochemicals from these plant materials used in our study. Some factors such as the extraction methods, plant part, geographical and environmental factors could have greatly contributed to such differences.

Farmers were observed to have the highest prevalence of corneal ulcers for both males and females. However, females were more infected with the corner ulcers and this could be due to the fact that they are involved in activities that require them putting hands and objects to the eyes. Some of the hands and objects could be contaminated and putting them to the eyes may result in ocular contamination[32]. Meena *et al*.[33] reported that agricultural workers were a greater proportion of corneal ulcer cases, this was consistent with our findings. The risks were linked to injury sustained form vegetative matter such as rice chaff, grasses, leaves, small stones and sand particles during harvesting especially. Our findings were also similar to the work carried out in a study from Bangalore India were approximately 45% of corneal ulcer patients were farmers followed by laborers, housewives

etc.[34] and another work by Ibanga et al.[35] who also observed that the cause of corneal ulcer was mainly trauma from agricultural sources. Farmers are in constant contact with farm tools and the vegetation more than the other groups involved in this study, and factors such as low education levels, delayed access to care, limited use of protective gear while in the farm pose as risk factors. Also, environmental factors such as dust, windblown particles, insect contact, crop residues are more common in the fields and these can carry fungi, bacteria and foreign particles which could cause corneal ulcers.

Bacteria isolated were *S.aureus(62.82%), S. viridans(35.89%), P. aeruginosa(49.35%), Streptococcus pyogenes(36.53%), Bacillus cereus(28.84%), Proteus mirabilis(30.76%) and klebsiella pneumoniae(44.23%)*. *S. aureus* was the most common and most isolated bacteria which is a Gram positive bacteria and a normal flora of the various body parts including the skin, followed by Pseudomonas aeruginosa which is a Gram negative pathogenic bacteria. This was in agreement with the work of Maurya *et al*.[36] who worked on Clinical and Microbiological Spectrum of Corneal Ulcer in Eastern Region of Uttar Pradesh and also with the findings of Roy *et al*.[37] who did a 6 year study on Changing Trends in Fungal and Bacterial Profile of Microbial Keratitis at a Tertiary Eye Care Centre, they both found *S.aureus* and *Pseduomonas aeruginosa* to be the major leading microbes in their studies. *S. aureus* is a leading cause of bacteria keratitis and can produce rapid and aggressive infections of the cornea. It commonly colonizes the eye lids and conjunctiva and can easily become opportunistic when there are breaks in the corneal epithelium due to trauma, dry eye and surgery. This can lead to stroma , causing pain , discharge, rising from the production of toxins and enzymes that accelerate tissue destruction[38]. *P. aeruginosa* has been recorded by researchers to be an aggressive corneal pathogen that raises an ophthalmic emergency. It possesses enzymes, exotoxins and motility that allow rapid stromal invasion and necrosis[39]. Streptococci are less common than staphylococci but can cause indolent, crystalline or slowly progressive keratitis and have been implicated in postoperative infections of the cornea; if untreated, can threaten vision. *Streptococcus pyogenes* and *Proteus mirabilis* have also been implicated in corneal ulcers and have been found to post-surgical infections, trauma or severe ocular surface diseases[40]. *Proteus mirabilis* though an uncommon corneal pathogen, has been associated with individuals with poor ocular surface integrity and contact lens wearers[41]. *Klebsiella pneumonia* is also another rare organism found, but if seen indicates serious cornea infections. It has been associated with systemic infections such as bacteraemia seeding the cornea with some strains producing aggressive necrotizing ulcers[42]. *Bacillus cereus* is an environmental organism often linked to trauma with vegetative matter or soil causing rapid tissue destruction and high risk of perforation. It has also been implicated in vision threatening keratitis with rapid necrosis and reports on cases has shown rapid progression and poor outcomes if not treated early[43]. Fungi isolated were A. flavus (63.46%), *A. fumigatus* (58.97%), *Candida albicans* (60.25%), *Fusarium solani* (48.71%), *Aurobasidium* spp (42.30%) and *Alternaria spp*(36.53%). It was observed that *Aspergillus flavus* had the highest prevalence followed by *candida albicans* which is consistent with systematic reviews reporting fungi account for a variable but large fraction of keratitis diseases[44]. *A flavus* is an environmental microbe that is mostly isolated from cases of fungi keratitis worldwide, its spores are ubiquitous in the soil and on vegetative materials, ocular trauma associated with plant materials is a well-documented risk factor for cornea infections its effect is seen as it produces extracellular enzymes and toxins capable of degrading stromal tissue leading to necrosis and corneal thinning[45]. *Aspergillus fumigatus* on the other hand acts similar to *A. flavus* and is found on plant materials hence associated with ocular trauma found among agricultural workers[46]. *Fusarium solani* is another leading cause of fungi keratitis globally and is known for its aggressiveness and poor response to antifungal therapy, it can lead to perforations if not handled early[47]**.** *Aureobasidium spp* and*Altarnaria spp* are found in soil, wood and damp floor environments, they induce suppurative keratitis with infiltrates resembling those caused by filamentous fungi[48].

The antimicrobial activities of the extracts , showed that at low concentrations (25% and 50%) the ethanol extracts of *Garcinia kola* seed, *Cocos nucifera* nut and *Dacryodes edulis* nuts extracts had little or no inhibitory characteristics on both the bacteria and fungi isolates, but at 75% and 100% concentrations, zones of inhibitions were observed and when this was statistically significant when compared to the positive controls used in the study (Amikacin for bacteria; Amphotericin B for fungi). Research has shown that sufficient bioactive compound concentration is needed to reach bactericidal or fungicidal thresholds[49]. These findings from our studies based on concentration effect at 100% was similar to many research studies at same concentration, *Garcinia kola* has been reported to inhibit S. aureus and other pathogens in a dose dependent manner and showed measurable zones of inhibition only at higher extract[16,50]. Again, methanol and ethanol extracts of *Cocos nucifera* have been repeatedly reported to inhibit *S. aureus P aeruginosa* and *Candida spp* (19) and studies on the antibacterial activity of the seed and pulp extracts against *D edulis* also showed wide zones of inhibition at 100% concentration[51]. The broad zones of inhibition observed during this research are attributed to the fact that these plant seeds contained a mixture of these phytochemicals that carry out their activity against these bacteria and fungi cell differently, ranging from membrane disruption, to enzyme inhibition, metal chelation, and to interference with cell- wall synthesis, hence giving it a firm grip on a wider group of organisms in-vitro[49].

The ability of these plant seed extracts to induce healing (Table 7-9) compared to the conventional medicine within five days shows that these plants have potentials that could help tackle antimicrobial resistance[45]. Our findings align with the established broad spectrum antimicrobial and wound healing properties of *Gacinia kola* which is attributed to the bioactive compounds such as flavonoids and tannins[52]. Study by Okoye *et al*.[53] reveals that *G.kola* extracts fastens epithelial regeneration and reduce microbial load in experimental keratitis models. *Cocos nucifera* showed significant healing properties from day four and complete improvement by day five. Its application in ocular infections have shown that it promotes wound contraction and epithelial recovery, consistent with the progressive healing seen in both bacterial and fungal ulcers[54]. Similar healing reaction was seen with *Dacryodes edulis* which has moderate phytochemicals and studies have reported it efficacy in treating resistant bacterial infections and in modulating inflammation, hence its potency in corneal ulcer[55]. Since corneal ulcers are particularly prevalent in tropical regions where crude agricultural practices are carried out and these plant seeds are indigenous, their use as affordable, locally available therapies could have a considerable public health impact.

V. Conclusion

The findings provide strong evidence that locally available medicinal plants hold valuable potential in ocular therapeutics. Their demonstrated efficacy supports the integration of phytomedicine into eye care as a sustainable, affordable, and culturally relevant option. This approach not only broadens treatment alternatives for corneal ulcers but also addresses the pressing challenge of antimicrobial resistance, offering a promising pathway toward improved eye health outcomes, particularly in resource-limited communities.

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