PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITIES OF *Carica papaya* L. AND *Azadirachta indica* L. AGAINST SOME BACTERIAL ISOLATES FROM BARBERING TOOLS

Supported by tetfund TERTIARY EDUCATION TRUST FUND

Adomi, Patience O.
Department of Microbiology, Delta State University, Abraka, Nigeria
padomi.adomi07@gmail.com

Received: February 19, 2021 **Accepted:** May 16, 2021

Abstract: The study was carried out to investigate the Antibacterial, qualitative and quantitative phytochemical screening of aqueous and ethanolic extracts of *Carica papaya* L. seeds and *Azadirachta indica* L. leaves against isolates from barbering salon tools. Isolation and identification of isolates from barbing salon was done using standard methods. Susceptibility testing, preliminary qualitative and quantitative phytochemical constituents were determined using standard methods. Bacteria isolated were *Staphylococcus aureus, Streptococcus* spp. *Escherichia coli* and *Pseudomonas aeruginosa*. Aqueous and ethanol extracts of *C. papaya* and *A. indica* were potent against the organisms at various concentrations. Extracts were potent at 200 mg/ml against *S. aureus* and *Streptococcus* spp. which are agents of folliculitis. *Escherichia coli* was the most susceptible organism showing zone of inhibition of 18±0.58 mm for aqueous *C. papaya* and 25±0.58 for *Azadirachta indica* ethanol extracts at 200 mg/ml. Qualitative and quantitative phytochemistry showed presence of tannins, saponins, cardiac glycosides, flavonoids in all extracts. Anthraquinone and alkaloid were absent in *Carica papaya* aqueous leaf and *Azadirachta indica* ethanol extracts. Plants could be useful source of treating bacterial diseases associated with barbering and barbering salon tools and also could be introduced into hair care preparations.

Keywords: Antibacterial, barbering tools, *Azadirachta indica*, *Carica papaya*, phytochemical screening

Introduction

The skin is a complex living ecosystem, harboring microbial communities. Its highly variable properties and the effect of intrinsic and extrinsic factors creates unique microenvironments where niche-specific microorganisms survive. Hair,as part of the skin, supports its own microbial habitat which is also intra and inter-personal variable (Brintac *et al.*, 2018). Hair is made up of alpha-keratin. The human hair shaft is a keratinized fibrous tissue which grows out of follicles beyond the surface of the epidermis. The human body is covered in follicles that produce thick terminal and vellus hair excluding the soles of the feet and palms of the hands. A cross section of the hair shaft showed three zones: the cuticle, cortex and medulla (Jablonski, 2006). The cuticle protects the inner structure of the hair which consists of several layers of flat, thin cells laid out like roof singles, the cortex provides the hair, its structure which contains keratin bundles in cell structures that remains roughly rod like. The medulla is for hair elasticity and open area at the fibre centre. The normal functions of hair include protection of inner tissues and organs, regulating temperature of the body, and quickening evaporation of perspiration; It also serve as a sense organ (O'Rahilly *et al*., 2004).

Human being love healthy hair due to its normal functions, social role and indication of status. Healthy hair implies health and youth while colour and texture can representsc ethnic ancestry. Hair on the face (facial hair) indicates puberty in men. Presence of white hair is a sign of age or genetics and male pattern baldness is a sign of age (Ashby, 2016; Hielscher, 2016).

Hair care involves cosmetology and hygiene of hair including hair on the scalp, facial hair (moustache and beard), pubic hair and other body hair. Hair care differs according to person's culture and the physical characteristics of one's hair. Hair may be coloured, trimmed, shaved, plucked, or otherwise removed with treatments such as waxing, sugaring, and threading. Barbering operations include cutting, hair dying, scalp and face massaging, nail trimming, manicure, pedicure, and shampooing. Barbering operations are carried out by barbers who sometimes use unsterile tools. Hair hygiene is important to prevent infections like ringworm, dandruff and other infections associated with barbering operations. Staphylococcal infections, Scabies, hepatitis and human immunodeficiency virus are transmitted through barbering tools (Amir and Raymond, 2005).

Plants have been known to possess healing properties. The healing properties are due to secondary metabolites produced by medicinal plants. It is in this respect that two plants *Carica papaya* Linn seeds and *Azadicrachta indica*, Linn leaves as shown in Plates 1 and 2 were selected to investigate the antibacterial effect on bacterial isolated from barbering salon tools.

Plate 1: *Azadicrachta indica*, Linn

Plate 2: ***Carica papaya*** **Linn seeds**

Materials and Methods

Collection of samples

The samples for research were randomly collected from barbering salons in Abraka, Delta State. Barbering tools such as combs, brushes, aprons were swabbed with sterile swab stick moistened with sterile normal saline. For the clipper, the swab stick was carefully rotated over the inner surface and over the surface of the cutting edge of the clipper (Gholamereza, 2009). Samples were collected after due approval from the owner of barbering salons.

Isolation and identification of test isolates

The labeled swab sticks were transported quickly to the Microbiology laboratory, Delta State University Abraka and streaked directly into nutrient agar and MacConkey agar prepared according to the manufacturer's instructions and incubated at 37ºC for 24 h. Pure culture was obtained by sub culturing distinct colonies into nutrient agar. Identification of culture was carried out conventionally using cultural, morphological characteristics and biochemical tests (Cheesbrough, 2004).

Collection and extraction of plant materials

Two plants were used for this study. These were *Azardiractcha indica* leaves and *Carica papaya* seeds. The plants were collected from the wild and identified at the Botany laboratory in Delta state University, Abraka. Plant samples were air dried within two weeks at room temperature. Pulverised plants materials were extracted using water and ethanol. One hundred grams (100 g) of each sample was introduced into flat bottom flask containing 500 ml of distilled water or 70% ethanol. The flasks were shaken to mix, and left to stand for 48 hours, with occasional swirling. Filtrates were obtained by using Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator. The solid crystal/oily extract obtained were weighed and then kept in sterile labeled bottles and stored at 4ºC until required.

Antibacterial activity of crude extracts

Standardized inoculum was prepared by transferring an overnight culture into Muller hinton broth. The Overnight cultures were diluted appropriately by adding normal sterile saline to the density of the inoculum and standardized then by comparing with 0.5 McFarland standard of barium sulphate solution.

Agar well diffusion technique was used for antibacterial activity determination. Exactly 0.2 mL of appropriate concentration of extract was introduced into wells bored on the agar surface after inoculation. Plates were incubated at 37ºC for 18-24 h. Two plates were inoculated for each dilution and control plates were prepared using same solvent used for plant extraction.

Mininum inhibition concentration determination (MIC)

The MIC for extract was determined by broth dilution method in Mueller Hinton Broth (CLSI, 2012). The initial concentration of extract 200 mg/ml was diluted double fold by transferring 5 ml of the sterile plant (stock solution) into 5 ml of sterile Muller hinton broth to obtain 100 mg/ml concentration. The process was repeated serially to obtain other dilutions 50, 25, 12.5, 6.25. Exactly 0.1 mL of standardized bacterial cell suspension was then inoculated into each test tube and incubated at 37ºC for 24 h. Turbidity was taken as growth. The lowest concentration of the extract with no growth of the test organism indicated the minimum inhibition concentration. Negative and positive controls were done. The last tube which showed no growth in MIC was plated out and incubated; the minimum bactericidal concentration was the tube that did not show any growth.

Phytochemical analysis of plant extracts

The qualitative and quantitative phytochemical screening of plants were carried out according to Trease and Evans (1989). Alkaloids and tannins, were assayed by methods described by Kumar *et al*. (2007); while cardiac glycosides, saponins and flavonoids as described by Odebiyi and Sofowara (1978) and Evans (2006).

Results and Discussion

Table 1 shows the culture, morphology and biochemical characterization of the bacterial isolates which included *Staphylococcus aureus*, *Streptococcus* spp, *Escherichia coli* and *Pseudomonas aeruginosa*. Fig. 1 shows the antibacterial activity of *C. papaya* seed aqueous and ethanol extracts. *Escherichia coli* was the most susceptible having zone of inhibition of 18±0.58 mm for aqueous extract at 200 mg/ml and *Pseudomonas aeruginosa* was the least sensitive (10 ±0.58 mm) at that same concentration. *Staphylococcus aureus* and *P. aeruginosa* showed moderate sensitivity to ethanol extract while *E. coli* (7.00) was the least sensitive.

Table 1: Cultural, morphological and biochemical characteristics of the bacterial isolates

Cultural	A1	A2	A3	A4
Elevation		Convex	Low convex	
Margin	Entire	Entire	Entire	Entire
Colour	Cream	Milkish white		Green
Shape	Round	Round	Round	Round
Size	Small	Medium	Small	
Morphological				
Gram staining	+	+	-	-
Cell type	Cocci	Cocci	Rod	Rod
Cell arrangement	Cluster	Chains	Single	Single
Biochemical characteristics				
Catalase	+	-	+	+
Oxidase	-	+	-	+
Coagulase	+	+	-	+
Urease		-	-	
Indole	+	-	+	
Citrate	-	+	-	-
Sugar fermentation				+
Glucose	+	+	+	+
Sucrose	+	-	-	+
Lactose	+	-	+	-
Isolates	*Staphylococcus aureus*	*Streptococcus* sp	*Escherichia coli*	*Pseudomonas aeruoginosa*

Figure 2 describes the antibacterial activity of *Azardirachta indica* to bacterial isolates. *E. coli* was the most susceptible organism to ethanol extract with zone of inhibition of (25±0.58) and others showed varying sensitivity; *P. aeruginosa* (23±0.58) and *Streptococcus* spp. (22±0.58), respectively. Aqueous extract was equally potent against the organisms at various concentrations considered. The minimum inhibitory and bactericidal concentrations are presented in Table 2. The MIC ranged from 50 – 200 mg/ml and MBC was within 25 to 200 mg/ml for the extracts. Tables 3 and 4 show the preliminary qualitative and quantitative constituents of the phytochemical of the various extracts. Both extracts derived from *C. papaya* seed contained tannins, saponins, cardiac glycosides, flavonoids, terpeniods, steroids while alkaloids and anthraquinones were not present in aqueous of same plant but present in ethanol crude extract. Similar result was obtained for *A. indica*. Except for *A. indica* ethanol crude extract where alkaloids and anthraquinones were absent.

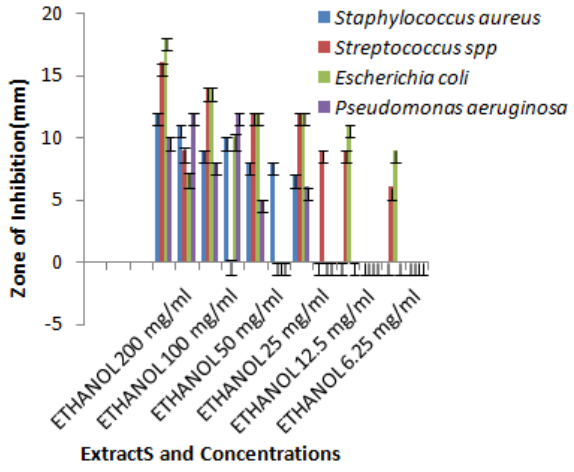

Fig. 1: Antibacterial activity of *Carica papaya* extracts against isolates from Barbering tools

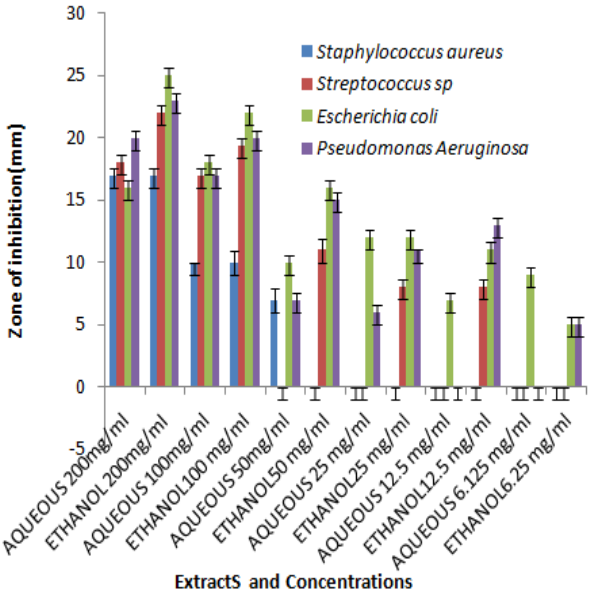

Fig. 2: Antibacterial activity of *Azadirachta indica* extracts against isolates from barbering tools

Table 2: The minimum inhibitory and maximum bactericidal concentration of extracts

Isolates	Mg/ml MIC				Mg/ml MBC			
	CA	CE	AA	AE	CA	CE	AA	AE
Sta. aureus	100	100	100	50	200	200	200	200
Stre. sp	200	200	50	50	200	200	100	100
E. coli	100	-	50	25	100	-	50	50
P. aeroginosa	50	100	50	100	100	100	100	100

CA=*Carica papaya* Aqueous, CE =*Carica papaya* Ethanol extract, AA= *Azadirachta indica* aqueous, AE- *Azadirachta indica* ethanol extract

Table 3: Qualitative phytochemical results of *Carica papaya* and *Azadirachta indica*

Qualitative phytochemistry	CA	CE	AA	AE
Tanins	++	+	+	+
Saponins	++	+	++	+
Flavonoids	++	++	+	+
Terpenoids	++	++	+	+
Cardiac glycosides	++	++	+	+
Alkaloids	-	+	++	-
Anthraquinones	-	+	-	-
Steroids	+++	++	+	+

CA=*Carica papaya* Aqueous, CE =*Carica papaya* Ethanol extract, AA= *Azadirachta indica* aqueous , AE- *Azadirachta indica* ethanol extract

Table 4: Quantitative phytochemical results of *Carica papaya* and *Azadirachta indica*

Quantitative phytochemistry	CA (%)	CE (%)	AA (%)	AE(%)
Tannins	3.10	3.40	2.30	2.40
Saponins	2.40	3.20	2.10	2.30
Flavonoids	3.10	3.90	2.60	2.30
Alkaloids	-	2.10	3.10	2.30
Phenols	1.20	1.20	3.40	2.00

CA=*Carica papaya* Aqueous, CE =*Carica papaya* Ethanol extract, AA= *Azadirachta indica* aqueous , AE- *Azadirachta indica* ethanol extract

Terpenoids, saponins, flavonoids, tannin, cardiac glycosides, were present in varying quantities. Quantitive phytochemistry results reflected the picture of the qualitative. For *Carica papaya* aqueous extract, the flavonoids and tannins were high (3.1%), phenols was lowest, *C. papaya* ethanol extract constituents showed the highest content of 3.9% for flavonoids and lowest for phenol at 1.2%. *Azadirachta indica* showed 3.4% for phenols and lowest (2.1%) for saponins. *A. indica* ethanol extract was 2.4% as the highest, while 2.0% for phenols.

The cultural, morphology and characterization of isolates from barbering tools showed four different isolates; *Staphylococcus aureus, Streptococcus sp., Escherichia coli* and *Pseudomonas aeruginosa*. Evbuomwan *et al*. (2018) and Eribo *et al*. (2017) isolated similar organisms from barbering tools except that *Bacillus* sp., *Enterococcus* sp. and *Enterobacte*r sp, and *Klebsiella pneumoniae*, were isolated in their study but absent in this study.

Folliculitis is an inflammation of the follicles of the hair. It can occur due to infection, chemical irritation and physical injury. Bacterial folliculitis is caused by *Staphylococcus aureus,* and less often coagulase negative staphylococci. The predisposing factors to bacterial folliculitis are frequent shaving, waxing or other forms of depilation (Amanda, 2016). Children, adolescents and young males are often affected by bacterial folliculitis. Areas infected include scalp, beard areas, axilla and extremities. Diseases on scalp and hair are often transmitted through contaminated barbering tools thus the very need to sterilize tools before used and after use for each client.

Methods employed by barber for decontamination of their tools have been reported to be ineffective and inadequate (Ebuara *et al*., 2020). Most barbers decontaminate their barber clippers using chemicals like methylated spirit, hydrogen peroxide, hypochlorite, petrol even kerosene. Chemical and flame are commonly used as well to decontaminate clippers. Previous study reveal that these methods employed by barber to decontaminate their clippers are not effective to destroy microorganism especially spores since the time of exposure is very short for the chemical, or chemical and flame to kill the organisms and spores (Emele *et al*., 2015). The isolation of bacterial from tools from these barbering salon showed vividly that the decontaminating agents and time are inadequate hence the presence of pathogenic bacteria on tools. Combs and brushes are scarcely disinfected between clients. These tools could be the source of inoculation of these organisms to clients and may result to various conditions such as folliculitis barbae, a type of folliculitis which affect beard area in men who shave and those who do not shave and deep seated folliculitis barbae called sycosis barbae (barber's itch) (Baron, 1996; Amanda, 2016).

The effects of two plants on isolates from barbering tools was also investigated *Azadirachta indica* (neem) leaves and *Carica papaya* (pawpaw)seeds. *Azardirachta indica* is used for hair care in Ethnomedicine. This plant, seeds and leaves are applied over hair as insecticides to kill lice (Muanya *et al*., 2019). The infusion of fresh leaves are applied to head for dandruff. The mixtures of seed and exuded sap from trees growing near water is massaged on bald head to promote hair growth (Punjani and Kumar, 2003). *A. indica* could be used against organisms associated with barbing tools. Previous study show that *A indica* has antibacterial activity against *Klebsiella* species and *S. aureus* (Quazi *et al*., 2014), thus agreeing with this study.

Carica papaya seeds have antibacterial activity and effective against *E. coli, Salmonella* and *Staphylococcus* infections. Fruits and seed extracts have antibacterial activity against *S. aureus, Bacillus cereus, E. coli*, and *P. aeruginosa* (Emeruwa, 1982). The seeds of *C. papaya* irrespective of its fruit maturity stages have antibacterial activity on gram positive and negative organisms which could be useful in treating infections (Jyotsna *et al*., 2014). So can be used by those who have bacterial infections associated with barbering tools.

The phytochemical constituents of seeds are different from leaves. Previous study showed the presence of alkaloids in water, ethanol and methanol extracts of *C. papaya* leaves (Adomi, 2018), contrasting the absence of alkaloids in water extract of seeds.

Qualitative screening of *A. indica* extracts showed the presence of steroids, tannins, saponins, flavonoids, terpenoids, cardiac glycosides in both aqueous and ethanol extracts and alkaloids in only aqueous extract and no anthraquinone in both extracts These findings conform to the study of Nwali *et al*., (2018) which detected similar result in ethanol extract of *A. indica*, but in their research, cardiac glycocides was absent. Quantitative screening also showed similar result with Nwali *et al*., (2018), which showed flavonoids 2.60% in this research while 2.19% in theirs. These phytochemical constituents are responsible for the antibacterial activity. Ethanol extract of *C. papaya* contained alkaloids, saponins, tannins, flavonoids, terpenoids, cardiac glycosides, steroids and anthraquines. These findings conform to the findings of Delphin *et al.* (2020) which showed presence of tannin, saponin and phenols. However, aqueous extract lacked alkaloid and anthraquinones thus showing that ethanol is a better solvent for extracting the phytochemical constituents of C papaya seeds. While water is the better extractant of phytochemical constituents of *A. indica*

Conclusion

This study showed that *Staphylococcus aureus, Streptococcus* sp. *Escherichia coli* and *Pseudomonas aeruginosa* were isolated from barbering salon tools in Abraka. *Azadirachta indica*leaves, *and Carica papaya* seeds were potent against the bacterial isolates. The minimum inhihitory concentrations of *C. papaya* seeds extracts on *S. aureus* was 100 mg/ml, and 100 and 50 mg/ml respectively foraqueous and ethanol extracts of *Azadirachta indica* leaves. The minimum bactericidal concentrations was 200 mg/ml for the extracts for same organism. Phytochemical results showed the presences

of tannin, steroids flavonoids, cardiac glycosides terpeniods, alkaloids and anthraquinones in varying quantities but flavonoids was the most abundant in *Carica papaya* ethanol extract while tannins and alkaloid were highest in quantities in aqueous extracts. Phenols were the highest in *Azadirachta indica* aqueous extracts. Findings from this study show that these extracts could be used in treating bacterial diseases such as folliculitis and other disease associated with barbering and barbering tools. Further work on the separation and identification of component of plant responsible for antibacterial activity should be investigated.

Conflict of Interest

The author declares that there is no conflict of interest reported in this work.

References

Adomi PO 2018. The antibacterial, phytochemical and synergistic activity of crude leave extracts of *Carica papaya*, *Cassia alata*, *Jatropha curcas* and *Manihot esculenta. Devtal. J. Sci. and Techn. Res.*, **7**(2): 38-47.

Amanda O 2016. Bacterial folliculitis on DermNet New Zealand Trust. https://dermnetnz.org/topics/microorganisms-found-on-the-skin/acaessed june 2020

Amir K & Raymond AJ 2005. Barbering shaving and blood-borne disease transmission in developing countries. *South Afri. Med. J.*, 95**(**2): 94-96.

Ashby SP 2016. Archaeologies of Hair: The head and its grooming in ancient and contemporary societies. *Internet Archaeology*, 42. *doi*:*10.11141/ia.42.6*

Baron S 1996. Microbial Infection of Skin and nail. Medical Microbiology 4th ed Galveston (TX): University of Texas medical Branch Galveston https://www.ncbi.nlm.nih.gov/books/NBK8301/ Laboratory Practice in Tropical Countries. 2nd Edn., Cambridge University Press, Cambridge, United Kingdom.

Brintac L, Clarke TH Sing H, Greco C, Gomez A, Torrabbe MG, Frank B & Nelson KE 2018. Spartial and Environmental variation of the Human Hair Microbiota, *Scientific Report. www/nature/com/scientificreports 1-7.*

Cheesbrough M 2004. District Laboratory Practice in Tropical Countries, Part 2 second edition Cambridge University Press, pp. 45-67.

CLSI 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.

Delphin DV, Haripriya. R, Subi S, Jothi D & Thirumalai Vasan P 2020. Phytochemical Screening Of Various Ethanolic Seed Extracts, *World J. Pharmacy and Pharmac. Sci.*, Abstract.

Ebuara FU, Imarenezor EPK, Brown JTC, Aso RE, Obasi IC & Tyovenda ET 2019. Isolation and Identification of pathogenic microorganisms associated with Barbers Equipment in Wukari, Tariba State Nigeria. *FUW, Trends in Sci. & Techn. J.*, 5(1): 215-218.

Emele FE, Oyeka C & Ubajaka CF 2015. Ringworm Infetion in Nigeria: Investigating the role of Barber's in Disease Transmission. *Int. J. Public Health Res.* 3(2): 67-71.

Emeruwa AC 1982. Antibacterial substance from *Carica papaya* fruit extract. *Journal of Naural Product*, 45 (2): 123-7 DOI10.1027/np50020a012

Eribo OA, Ebiale IF, Oyedoh PO&Oshdin OS 2017. Bacteriological assessment of Barber's clippers in barber's shops in Ugbowo, Benin city. *Africa Scientists*, 18(40): 223-226.

Evans WC2006. *Trease and Evans Pharmacognosy*. 15th ed. WB Saunder, Toronto.

Evbuomwan L, Chukwuka DE & Ebiala FI 2018. Isolation of multidrug resistant Bcterial pathogens from human hair obtained from barbing saolns located in Benin City, Nigeria. *South Asian J. Res. in Microbio.*, 2(4): 1-7.

Gholamereza S, Nooshin T, Ali M, Touraj-Reza M & Ehsan S 2009. Bacterial contamination and resistance to commonly used Antimicrobials of Health care workers mobile phones in teaching Hospitals, Kerman, Iran. *Amer. J. Appl. Sci.*, 6(5): 806-810,

Hielscher S 2016. Because You're Worth It: Women's daily hair care routines in contemporary Britain", Internet Archaeology 42. http:dx*doi*:*10.11141/ia.42.6.13*.

Jablonski Nina G 2006. Skin: A Natural History. University of California Press. pp. 13–. ISBN 978-0-520-94170-0. Archived from the original on 23 April 2016. Retrieved 27 January 2019.

Jyotsna KP, Yashab K, Priyanka P & Harison M 2014. Antibacterial activity of seed and leaf extract of Carica Papaya var. Pusa dwarf Linn. *J. Pharmacy and Biol. Sci.*, 9(2): 29-37.

Kumar GS, Jayaveera KN, Kumar CKA, Sanja UP, Swamy BMV & Kumar DVK 2007. Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. *Tropical J. Pharmac. Res.*, 6: 717-723.

Muanya C, Akpunonu S & Onyenucheya A 2019. Scientist Validate more herbs for hair growth. The Guardian 18th April.

Nwali ON, Idoko A, Okolie JE, Ezeh, E, Ugwudike PO, Rita ON, Ezenwali MO, Odi I A, Ani PN & Okolie SO 2018. Comparative analysis of the phytochemical compositions of leaf, stem-bark and root of *Azadirachta Indica* (Neem). *Universal J. Pharmac. Res.*, 3(5): 46-50.

O'Rahilly R, Muller F, Carpenters & Swenson R 2004. *Basic Human Anatomy, A regional study of Human structure.* Dartmouth Medical School. http://www.dartmouth.edu/~humananatomy/index.html

Odebiyi OO & Sofowora EA 1978. *Phytochemical screening of Nigerian Medicinal Plants* II. *Lloyidia*, 41(3): 234-46.

Punjani BL & Kumar V 2003. Plants used in traditional Phytotherapy for hair care by tribals in Sabarkanthra district, Gujarat, India. *Indian J. Tradit. Knowl.* 2(1): 74-78.

Quazi RB Feroza P & Jalaluddin I 2014. Growth inhibitory effect of ethanolic neem leaves extract on *Klebsiella, Salmonella* and *Staphylococcus aureus. Bangladesh J. Pharmacol*, 9: 347-350.

Trease GE & Evans WC 1989. *Trease and Evans' Pharmacsognosy: A Physician's Guide to Herbal Medicine.* 13th Ed., Bailliere Tindall London, pp. 1-104.

APPENDIX

Types of extract and concentration	***Staphylococcus aureus***	***Streptococcus spp***	***Escherichia coli***	***Pseudomonas aeruginosa***
AQUEOUS 200mg/ml	.0	.56	.0	.67
ETHANOL 200 mg/ml	.32	.0	.0	.0
AQUEOUS 100 mg/ml	.66	.4	.4	.32
ETHANOL 100 mg/ml	.0	.0	.0	.00
AQUEOUS 50 mg/ml	.0	.2	.2	.0
ETHANOL 50 mg/ml	.0	.0	.0	.0
AQUEOUS 25 mg/ml	.0	.2	.3	.0
ETHANOL 25 mg/ml	.0	.0	.0	.0
AQUEOUS 12.5 mg/ml	.0	.0	.0	.0
ETHANOL 12.5 mg/ml	.0	.0	.0	.0
AQUEOUS 6.25 mg/ml	.0	.0	.0	.0
ETHANOL 6.25 mg/ml	.0	.0	.0	.0