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Antibacterial activities and phytochemical analyses of *Mangifera indica* L.(Mango) and *Aloe Barbadensis* Miller (Aloe vera) against isolates from hair dressing tools in Abraka, Delta State

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Abstract
Microbial contaminant of tools used in hair dressing saloon may pose potential health risks to the public as these may lead to microbial colonization and disease transmission. Plants produce bioactive compounds which could be useful in treating diseases contracted during hair care. The study aim was to determine phytochemical and antibacterial analyses of M. indica and A. barbadensis against microbial isolates from hair dressing tools in Abraka, Delta State. Bacteria were isolated and identified from hair dressing tools using cultural techniques. The effect of crude extracts from gel and leaves of plants on bacterial isolates from hair dressing tools were tested using agar well diffusion technique. Phytochemical analyses were done using standard methods. Results showed that ethanolic extracts were more active against hair dressing tools isolates, which included Staphylococcus aureus, Pseudomonas aeruginosa, Streptococus sp. Micrococcus sp. and Escherichia coli. Streptococcus sp. showed the largest zone of inhibition of 20mm at 200mg/ml of ethanol M. indica while preliminary phytochemistry of Mangifera indica leaf extracts showed the presence of tannins, saponins, flavonoids, cardiac glycosides and absence of alkaloids, anthraquinones and phlebatanins. Aloe vera gel showed presence of saponins, flavonoids, alkaloids and tannins. Quantitative phytochemisty for Aloe barbensisaqueous extract was 0.25% for tannins and 1.52% for alkaloids while that of Mangifera indica extracts were 2.30%, 2.45%, 2.20%, 2.50% and 2.60% while 2.80%, 2.30%, 2.20%, 2.10% and 1.40% for aqueous and ethanol respectively for tannins, saponins, flavonoids, alkaloids and phenols. Aloe barbadensis and M. indica crude leaf extracts contained bioactive compounds which could be used against bacterial isolates from hair dressing tools.

Keywords: Antibacterial, *Aloe barbadensis*, bacterial isolates, hair dressingtools, *Mangifera indica,* phytochemical analyses

Introduction
Beauty and hairdressing salons are grouped as personal establishments where services rendered sometimes pose health risks to their customers due to injury and infection transmission (Adeleye and Osidipo 2004; Barn and Chen, 2011). When rendering services, tools like razor, combs, hair and pin can accidentally pierce the skin of their customers. Diseases transmitted through tools used in beauty and hair dressing salon may include ring worm, impetigo, and dandruff (Mbajiuk a*et al*., 2014). Even hair may also be infected since

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the tools for beauty salon procedure are shared and not all hair dressers disinfect their tools between customers. Most hair dressers are ignorant of the fact that there is possibility of transmitting certain diseases when rendering their services (Susanna *et al*., 2015).

Mango (*Mangifera indica*) originated from tropical Asia (Ian, 2006). It is a canopy tree that grows to the height of 8-40m (Litz, 2009). The plant leaf is 15-45cm in length with variable size (Nandwani, 2006), simple and alternately arranged. The petioles varies in length from 1-12cm, always swollen at the base. Leaves appear in variable shapes, lanceolate, oblong, oval-lanceolate, obovate-laneolate, linear-oblong, or roundish oblong. Red, yellow and green leaves are seen in mango varieties, while the upper leaf surfaces are shiny (Nandwani, 2006).

The ethnobotanical use of mango leaves include; anti diabetic used in the form of decoction or powder, in Bangladesh burnt mango leaves are applied locally on burns and scalds (Parvez, 2016). Leaves have also been reported to be used for throat infection and hiccups, hemorrhages, kidney disease, wounds, burns and scalds in India (Khare, 2008; Khundare, 2016). Mango leaves are used in treating earache, vomiting, and inflammation (Ediriweera *et al*,. 2017).

Aloe barbadensis Miller is of the family Asphodelaceae (Liliaceae) family, which is a succulent pea-green coloured, perennial shrub plant. The triangular freshy leaves have serrated edges, yellow, tubular fruits and flowers which contains numerous seeds. Plant is found in arid regions of Africa, America, India and Asia. The leaf is made of three layers, the first is the inner gel, the middle layer of latex containing bitter sap and an outer thick layer of 15-20 cells that contains, sugars, enzymes, vitamins and fatty acids (Surjushe, *et al*., 2008). The study was carried out to investigate the antibacterial and phytochemical analyses of *M. indica* and *A. barbadensis* against microbial isolates from hair dressing tools. Below are the plates showing *Aloe barbadensis* Miller. (Aloe vera) and *Mangifera indica* L. (Mango)

Plate 1: *Aloe barbadensis* Miller. (Aloe vera)

Plate 2: *Mangifera indica* L. (Mango)

Materials and Methods

Collection of samples and extraction of Plant Materials

Samples for study were collected from hair dressing salons in Abraka, Delta State after consent by the hair dressers. Combs, brushes and dryers were swabbed with moistened sterile swab sticks, labeled and transported to the laboratory immediately. Plants investigated were collected from the wild in Campus 2, Abraka. Plants were identified in Botany Department of Delta State University, Abraka.

The methods of Karumari *et al*. (2014) and Mbaijuku *et al.* (2014) were adopted with a little modification for aqueous and ethanol extracts of Aloe vera respectively. Leaves of Aloe vera fresh ones were washed then air dried in the laboratory for 2 hours and cut lengthwise with sterile knife. The gel was removed then 20g of crude paste of gel was mixed with 100ml of water while for ethanol extract, 20g of aloe vera paste was mixed with 70 % ethanol. The aqueous and ethanol extracts were filtered with muslin cloth and evaporated to dryness after 24hrs. The dry powder of *Mangifera indica,* leaves was soaked in 100ml of water/ 70% ethanol and allowed to stand for 24 hours and filtered. The oily brown crude extracts obtained after heating filtrates at 60-80^{o}Cwere weighed and kept in refrigerator.

Bacteria identification and antibacterial susceptibility Testing

The swabs collected were streaked into nutrient and MacConkey agar prepared according to manufacturer's instructions and incubated at 37^{o}C for 24 hrs. Pure isolates obtained by further subculture were identified through cultural morphology Gram staining and various biochemical tests (Cheesbrough, 2000).

A loopful of standardized inoculum was spread uniformly on solidified Mueller hinton agar plates. Using sterilized cork borer (5mm), well was made on the seeded agar and 100µl of different concentrations were introduced into well and incubated. Duplicate plates were done for each extract while control plates contained solvent for extraction.

Minimum inhibition concentration determination (MIC).

The MIC for each extract was investigated using broth dilution method in Mueller- Hinton broth. Two hundred millilitres of crude extract was diluted double fold as 2ml of the sterile plant extract (stock solution) was introduced into 2ml of sterile Mueller Hinton broth to obtain 100mg/ml. Dilutions were carried out severally until 6.25mg/ml was obtained. Standardized bacterial cell suspensions (100µl) was inoculated into tubes and incubated at 37^{o}C for 24 hours. The test tube having the lowest concentration of extract without growth indicated minimum inhibitory concentration. Negative controls and positive controls were set up.

Phytochemical Analysis of Plant Extracts

Qualitative analysis

Qualitative phytochemical screening tests on extracts were carried out as described by Trease and Evans, (1989); Odebiyi and Sofowara, (1978) as previously reported (Adomi and Osey-Jovy, 2020).

Quantitative analysis

Alkaloid

Quantification of alkaloid was carried out by adopting Harborne (1973) method where 500mg of sample was mixed with 200ml of 10% acetic acid in ethanol, and allowed to stand for 4h. Concentrated ammonium hydroxide was added drop wise to filtrate which had been reduced to one quarter of the original volume by heating on a water bath. Ammonium

hydroxide was used to wash the precipitate formed and filtered. The residue which is the alkaloid was dried and weighed.

Saponin

The method described by Obadoni and Ochuko (2001) was adopted. Dry plant powder (20g) was added to 100ml of 20% aqueous ethanol, in a conical flask and heated to 55^{o}C on a water bath for 4h with continuous stirring. The mixture was then filtered. The residue obtained was re-extracted with another 20% ethanol (200ml), the combined extract obtained was reduced by heating, the concentrate was transferred in to a separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The ether layer was discarded while 60ml of n-butanol was added to the aqueous layer and then washed twice with 5% aqueous sodium chloride (10 ml) and dried to a constant weight. The percentage of the saponin was then calculated

Phenols

The experiment to determine the phenolic component was determined by heating fat free sample in 50ml of ether for 15min. One hundred milliliters of distilled water was mixed with 5ml of extract in a 50ml volumetric flask then 2ml ammonium hydroxide solution and 5ml of concentrated amyl alcohol was added. The sample was made up to mark and allowed to stand for half an hour for development of colour. Absorbance was measured at 505nm using a spectrophotometer (Ukpabi *et al*., 2013).

Tannin

Tannin content of plants was determined using Van-Burden and Robinson (1981) method. Fifty millilitres of distilled water was added to 0.5g of sample in a plastic 50ml bottle and shaken for 1hr in a mechanical shaker and then filtered into volumetric flask (50ml). Five millilitres of filtrate was pipetted into a test tube, 2ml of 0.1N HCl and 0.008M potassium ferrocyanide.,was then added and absorbance measured at 605 nm within 10mins.

Flavonoid

The modified method of Bohm and Kocipai-Abyazan, (1994) was used. The plant material (0.1g) was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. Mixture was filtered and heated to dryness in a crucible on a water bath until a constant weight was obtained.

Results And Discussion

Results

The antibacterial activities of *Mangifera indica* and *Aloe barbadensis* ethanol and water extracts on isolates from hair dressing saloon tools and phytochemical analyses of the plants were carried out. Results as presented in Table 1 are the isolates from hair dressing saloon at Abraka. Two gram negative bacteria; *Pseudomonas aeruginosa* and *Escherichia coli,* while three gram positive bacteria*: Micrococcus* sp, *Staphylococcus aureus,* and *Streptococcus sp*were isolated.

Table 1: Bacterial Isolates from Hair Dressing Salon Tools in Abraka

Gram Positive Bacteria	Gram Negative Bacteria
Staphylococcus aureus	*Escherichia coli*
Streptococcus spp.	*Pseudomonas aeruginosa*
Micrococcus spp.	

Table 2 shows antibacterial activities of Aloe vera gel. extracts. The aqueous extract was not potent against the organisms while the ethanol extract was potent at various concentration considered except the lowest concentration (6.25mg/ml). At 200mg/ml, which was the highest concentration determined, zone of of inhibitions were 11.00mm, 10.00mm, 9.00mm 9.00mm and 8.00mm for *Pseudomonas aeruginosa, Streptococcus* sp., *Escherichia coli*, *Staphylococcus aureus* and *Micrococcus* sp. respectively. For *Mangifera indica*, *Streptococcus* sp produced the largest inhibition diameter of 20.00mm for ethanol extract (Table 3). Minimum inhibitory concentration ranged from 12.5 to 100mg/ml for *M. indica extracts* while 50mg/ml for ethanol Aloe vera gel extract as shown in Table 4. Qualitative and quantitative analyses for plant extracts are presented in Tables 5 and 6. Aqueous extract of Aloe vera contained tannins, alkaloids, flavonoids and saponins, *Mangifera indica* leaves for both aqueous and ethanol contained, tannins, flavonoids, saponins, cardiac glycosides, but absence of alkaloids (ethanol) extracts, anthraquinone and phlebatanins. Quantitative constituents for Aloe vera gel shows that Aloe vera contained 1.52% alkaloids, 1.02% phenols, 0.72% flavonoids, 0.55% saponins, and 0.25% tannin. *Mangifera indica* showed varying content for alkaloids (2.50%; 2.10%), phenols (2.60%; 1.40%) and tannin (2.30%, 2.80%), for aqueous and ethanol respectively.

Table 2: Antibacterial Activity of Aloe vera gel. against Bacteria from Hair Dressing Tools from Abraka

	Diameter(mm)											
	200mg/ml		100 mg/ml		50 mg/ml		25 mg/ml		12.5 mg/ml		6.25 mg/ml	
Bacteria Isolates	A	E	A	E	A	E	A	E	A	E	A	E
Escherichia coli	0.00	9.00	0.00	8.00	0.00	8.00	0.00	6.00	0.00	5.00	0.00	0.00
Pseudomonas aeruginosa	0.00	11.00	0.00	6.00	0.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00
Staphylococcus aureus	0.00	9.00	0.00	9.00	9.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Streptococcus sp.	0.00	10.00	0.00	10.00	0.00	6.00	0.00	6.00	0.00	0.00	0.00	0.00
Micrococcus sp.	0.00	8.00	0.00	7.00	0.00	8.00	0.00	8.00	0.00	5.00	0.00	0.00

Key: AA= Aleo vera Aqueous, AE Aleo vera Ethanol extract, MA=*Mangifera indica* aqueous, ME= *Mangifera indica* ethanol

Table 3: Antibacterial Activity of *Mangifera indica* against Bacteria from Hair Dressing Tools from Abraka

	Diameter(mm)											
	200mg/ml		100 mg/ml		50 mg/ml		25 mg/ml		12.5 mg/ml		6.25 mg/ml	
Bacteria Isolates	A	E	A	E	A	E	A	E	A	E	A	E
Escherichia coli	5.00	19.00	5.00	18.00	3.00	12.00	0.00	7.00	0.00	7.00	0.00	3.00
Pseudomonas aeruginosa	4.00	15.00	8.00	10.00	2.00	13.00	2.00	2.00	5.00	0.00	0.00	5.00
Staphylococcus aureus	8.00	19.00	4.00	14.00	0.00	7.00	2.00	5.00	1.00	5.00	0.00	2.00
Streptococcus sp.	8.00	20.00	5.00	12.00	1.00	10.00	3.00	1.00	0.00	8.00	0.00	2.00
Micrococcus sp.	7.00	19.00	4.00	14.00	0.00	7.00	2.00	5.00	1.00	5.00	0.00	4.00

Key: AA= Aleo vera Aqueous, AE Aleo vera Ethanol extract, MA= *Mangifera indica* aqueous, ME= *Mangifera indica* ethanol

Table 4: Minmum Inhibitary Concentration of Extracts of Aloe vera and *Mangifera indica* Extracts

Bacteria	AA	AE	MA	ME
Escherichia coli	-	50	50	25
Pseudomonas aeruginosa	-	50	100	25
Staphylococcus aureus	-	50	50	12.5
Streptococcus sp.	-	50	50	12.5
Micrococcus sp.	-	50	100	12.5

Key: AA= Aleo vera Aqueous, AE Aleo vera Ethanol extract, MA= *Mangifera indica* aqueous, ME= Mangifera indica ethanol

Table 5: Qualitative Phytochemical Results of Aloe vera and *Mangifera indica* Extrats

Qualitative Phytochemistry	AA	AE	MA	ME
Tannins	+	ND	+	+
Saponins	++	ND	++	+
Flavonoids	++	ND	+	+
Terpenoids	ND	ND	++	+
Cardiac Glycosides	ND	ND	+	+
Alkaloids	++	ND	++	-
Anthrauinones	ND	ND	-	-
Streroids	ND	ND	+	+
Glycosides	ND	ND	+	+
Phlebatannins	ND	ND	-	-

Key: AA= Aleo vera Aqueous, AE Aleo vera Ethanol extract, MA= *Mangifera indica* aqueous, ME= *Mangifera indica* ethanol

Table 6: Quantitative Phytochemical Result of Aloe vera and *Mangifera indica* Extrats

Quantitative Phytochemistry	AA(%)	AE(%)	MA(%)	ME(%)
Tannins	0.25	ND	2.30	2.80
Saponins	0.55	ND	2.45	2.30
Flavonoids	0.72	ND	2.20	2.20
Alkaloids	1.52	ND	2.50	2.10
Phenols	1.02	ND	2.60	1.40

Key: AA= Aleo vera Aqueous, AE Aleo vera Ethanol extract, MA= *Mangifera indica* aqueous, ME= *Mangifera indica* ethanol

Discussion

Microorganisms isolated from combs, brushes and dryers in hairdressing saloons in Abraka were Staphyltococcus aureus, Streptococcus sp, E. coli, Micrococcus sp. and Pseudomonas aeruginosa. Previous researchers reported similar bacteria as contaminants of hair dressing tools. Staphylococcus aureus, Micrococcus sp., Bacillus spp., Serriatia spp., Citrobacter spp. Proteus spp. and Shigella spp. were isolated by Stanley, *et al* .(2019), while Mbaijuka *et al* (2014) reported S. aureus, Streptococcus sp and Micrococcus as contaminants of hair dressing tools in Umudike. Sources of these organisms in hair dressing tools may include skin, hair and environment coupled with the fact that most hair dressers do not decontaminant tools before being used for their clients. Previous study using questionnaire survey indicated that few hair dressers 8(34.8%) out of 23 (100%) sanitize their combs and brushes between customers and only 1(4.3%) sanitize tools after use (Adomi, 2020)

Hair dressing saloon may pose possible sites where microorganism could be transmitted through communities especially among the women who sought for services of hair dresser The need for hair dressers to sanitize or decontaminate their tools cannot be overemphasized.Microbial load of hair dressing tools (combs and brushes) most always are heavy as reported by previous studies (Mbajiuka *et al*. 2014; Stainley *et al*., 2019) though not shown in this study. These heavy microbial load clearly indicates that tools are not always decontaminated hence the heavy load. The bacteria isolated in this present study are potential pathogens. Staphylococcus and Streptococcus species are known to cause diseases such as impetigo, boils, erythrasmas and folliculitis (Baron, 1996). Pseudomonas aeruginosa cause green nail syndrome where the nail plate becomes discoloured. This could be a potential risk to hair dresser themselves who immerse their hands in water often when carrying out their services.

Aloe vera gel ethanol extract was more efficacious than aqueous extract in this study as shown by the zone of inhibition produced by this extract. This finding is in line with previous report which showed that ethanolic extract was the most active compared with hexane, ethyl acetate, and petroleum ether extracts (Thirrupplathi *et al*.,2010). Also, in their study, E. coli was resistant to the aqueous extract but sensitive to ethanolic extract. The presence of tannin, saponins, alkaloids and flavonoids in aqueous extract also conform to the findings of Karumari, *et al*.(2014).The aqueous extract did not have antibacterial effect against any of the bacteria isolated from hair dressing tools in this study. In the same vein, ethanolic extract of M. indica was more potent than aqueous extract. This also conform to previous report which showed that ethanolic extract was very active compared to aqueous and chloroform extracts against bacterial isolates (Mohammed *et al*., 2016). Activity of these crude extracts on hair dressing isolates showed that various phytochemical constituents as shown in Tables 5 and 6 are capable of inhibiting growth of isolates and as such could be used against bacterial isolated from hair dressing salon tools.

Conclusion

This study showed that ethanolic extracts of Aloe vera gel and *Mangifera indica* leaves were potent against isolates from hair dressing salon tools. Quantitative constituents of extract showed that Aloe vera gel contained higher concentration of alkaloids than other phytochemical compounds. Similarly, quantitative phytochemical analysis of *M. indica* indicated that tannins and phenols were highest in ethanol and aqueous extracts respectively. Aloe vera gel and *Mangifera indica* leaves could be used as antibacterial agents against bacteria isolated from hair dressing tools at 50mg/ml and 12.5mg/ml respectively for Aleo vera and *M. indica*.

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