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Ethnobotanical Plants Used for Oral Pathogens among Esan Tribe in Esan West Local Government of Edo State

E.O. Oshomoh*[1], M. Idu[2] and K.E. Ehimuan[3]

[1]Department of Science Laboratory Technology, University of Benin, Benin City. Nigeria
[2]Department of Plant Biology & Biotechnology, University of Benin, Benin City. Nigeria
[3]Department of Pharmacology & Toxicology, University of Benin, Benin City, Nigeria

*Corresponding author Email: emmanuel.oshomoh@uniben.edu Tel: +234 (0) 805 545 2141

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ABSTRACT: Therapeutic plants have kept on drawing in consideration worldwide in the quest for powerful antimicrobial drugs that can battle resistant pathogens that have rendered numerous conventional medications out of date in the treatment of diseases. As such this study was carried out to evaluate the ethnobotanical survey of medicinal plants used for oral pathogens among Esan tribe in Esan West Local Government Area of Edo State. A total of four medicinal plants were collected and identified as follows: *Harungana madagascariensis, Jatropha curcas, Aframomum melegueta* and *Plumbago zeylanica.* They were separately cut into smaller pieces and air-dried for two weeks and at ambient temperature under the shade, grinded into powder, weighed and stored in an air tight container for further use. The quantitative phytochemical analysis of these medicinal plants used in the different Local Government Area revealed that alkaloids range from 1.8±0.31 – 10.0±0.48, carbohydrates (0±0.00 - 9.2±0.62), glycosides (6.9±0.31 - 8.6±0.72), steroid (0±0.00 - 4.8±0.40), terpenoids (0±0.00 - 4.2±0.66), saponins (1.2±0.50 - 8.6±0.51), phytosterols (2.2±0.48 - 5.7±0.19), triterpenoids (1.8±0.14 - 4.2±0.23), phlobatannins (2.5±0.08 - 4.5±0.78), resins (0±0.00 - 8.9±0.60), phenols (2.1±0.57 - 6.8±0.02), tannins (0±0.00 - 7.6±0.13), flavonoids (5.9±0.41 - 7.9±0.48) and anthraquinones (0±0.00 - 8.3±0.38). The antioxidant activity of the different plant extracts also shows that at 1000 µg/ml concentration values ranged from 40.1±3.29 - 45.5±3.10, at 500 µg/ml (35.4±2.70 - 37.9±2.52), at 250 µg/ml (22.8±2.35 - 29.5±2.36), at 125 µg/ml (12.4±1.31 - 18.1±1.80), at 62.5 µg/ml (5.2±0.48 - 9.4±0.78) and at 31.25 µg/ml (1.0±0.05 - 4.5±0.73). The antibacterial activity screened from the zone of inhibition indicate that the various plant extract concentrations (100, 80 and 60 mg/ml), 100 mg/ml showed the high degree of inhibition in descending order among the organisms, *E. coil* (11.00±2.55 - 13.00±2.26), *S. aureus* (10.50±2.31 - 13.00±2.42), *Streptococcus* sp. (10.50±2.62 - 12.00±2.23), *P. aeruginosa* (10.02±2.60 - 12.05±2.73), *P. mirabilis* (11.00±2.32 - 12.00±2.66) and *K. pneumonia* (10.40±2.65 - 13.10±2.81). This study, therefore advocated further research to understand the mechanism behind this wide range of antimicrobial activities.

Keywords: Ethnobotanical, Antimicrobial, Phytochemical, Antioxidant, Oral pathogens

Introduction

Herbalism remains the study of botany and use of plants intended for medicinal purposes or for supplementing a diet. Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today (Adams *et al.,* 2006). Modern medicine recognizes herbalism as a form of alternative medicine, as the practice of herbalism is not strictly based on evidence gathered using the scientific method. Modern medicine makes use of many plant-derived compounds as the basis for evidence-based pharmaceutical drugs. Although phytotherapy may apply modern standards of effectiveness testing to herbs and medicines derived from natural sources, few high-quality clinical trials and standards for purity or dosage exist (Adaramola *et al.,* 2012).

Harungana is a genus of flowering plants within the St. Johnswort family, Hypericaceae. It comprises only two species, *Harungana madagascariensis* and *Harungana montana*. The haronga, is a small-sized bushy tree that usually ranges 4 m to 7 m in height, but sometimes it can grow up to 25 meters. The branches stem out from a cylindrical truck. Its crown appears to be golden-green color (Adetutu *et al.,* 2011). *Harungana*

madagascariensis can be used in various ways. For example, *H. madagasacariensis* is a source of firewood and is used in the production of charcoal. The seeds *Jatropha curcas* contain around 20% saturated fatty acids and 80% unsaturated fatty acids, and they yield 25–40 % oil by weight (Curniawan *et al.*, 2021). The root decoction of *Jatropha curcas* is used for the treatment of eczema, scabies, ringworm, gonorrhea, dysentery, diarrhea, and the oil extract from the roots is used as an antihelmeintic agent (Das *et al*., 2018).

In African folk medicine, Aframomum species are used for alleviating stomach ache and diarrhea as well as hypertension, as an aphrodisiac, and against measles and leprosy. They are also taken for excessive lactation and post partem hemorrhage and are used as a purgative, galactogogue and anthelmintic, and hemostatic agent (Fasakin *et al*., 2017).

P. zeylanica (Ceylon leadwort, doctorbush or wild leadwort) is a popular medicinal herb throughout Africa and Asia. It has been used as a remedy for skin diseases, infections and intestinal worms viz. leprosy, scabies, ringworm, hookworm, dermatitis, acne, sores and ulcers since time immemorial (Shekar *et al*., 2021).

Dental abscess is a localized collection of pus associated with a tooth. The most common type of dental abscess is a periapical abscess, and the second most common is a periodontal abscess. In a periapical abscess, usually the origin is a bacterial infection that has accumulated in the soft, often dead, pulp of the tooth (Chizzali and Beerhues, 2012). This can be caused by tooth decay, broken teeth or extensive periodontal disease (or combinations of these factors).

Therapeutic plants have kept on drawing in consideration worldwide in the quest for powerful antimicrobial drugs that can battle resistant pathogens that have rendered numerous conventional medications out of date in the treatment of diseases. Therefore numerous medications utilized in medicine are acquired from plants. This study will evaluate the ethnobotanical plants used for oral pathogens among the Esan tribe in Esan West Local Government of Edo State.

Materials and methods

Collection of samples: The plants were collected from Esan West Local Government Area of Edo State. The plant samples were identified in the herbarium by Prof. M. Idu of the Phytomedicine Unit in the Department of Plant Biology and Biotechnology, University of Benin as *Harungana madagascariensis* (UBH-G615), *Jatropha curcas* (UBH-W273), *Aframomum melegueta* (UBH-X518) and *Plumbago zeylanica* (UBH-H441).

Sample preparation: The samples were cut into smaller piece and dried under shade for weeks and at ambient temperature under the shade. The dried plant was latter oven dried at 45 °C to disintegrate the lipid wax and then prepared for grinding. The dried sample was then pulverized using a *Thomas Willey* milling machine in the Department of Pharmacognosy, University of Benin. The powdered sample was weighed and stored in an air-tight container for further use.

Extraction of sample: 200 g of the pulverized plant samples was weighed into an extracting bottle separately and was cold extracted in 2 L of distilled water for one day (24 h) with occasional shaking at intervals. After 24 h, it was filtered with sieve cloth to collect the filtrate.

Microbial strains: The following microbial identified strains (*Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Streptococcus* sp.) were collected and transported with sterile sealed container from the University of Benin Teaching Hospital to the laboratory and kept in a refrigerator till needed for use. Before use, the microbial strains were inoculated in nutrient broth at 37 °C for 24 h in the incubator.

Preparation of culture media (nutrient agar): The medium was prepared by suspending 14 g of the agar powder in 450 ml of sterilized deionized water in a conical flask and stirred to dissolve properly. The conical flask was covered with cotton wool and aluminum foil paper and autoclaved at 121 °C for 15 min. The medium was allowed to cool before pouring into plates aseptically in the required amount. The plates were covered and allowed to solidify.

Determination of antimicrobial activity: Three levels of concentrations 60, 80 and 100 mg/ml of the extract were prepared for this test. The bacteria and fungi cultures were maintained on nutrient agar medium and potato dextrose agar medium respectively.

Agar well diffusion method: A loopful of each strain was collected into 15 ml of distilled water in a clinical bottle to activate the organisms. The activated organisms were streaked on the surface of appropriate media by using a sterile cotton swab sticks then sterile cork borer was used to punch wells (10 mm in diameter) in the cultured media. Each concentration of the plant extract was introduced into independent wells. Plates were incubated at 37 °C for 24 hours. The active extract showed zones of inhibition, measured using a meter rule to measure 4 points across the zone and the average diameter recorded.

Qualitative analysis of powdered sample

Test for Alkaloids: The method of Odebiyi and Ramstard (1978) was used. One (1) ml of hydrochloric acid was added to 3 ml of extract in a test tube. The mixture was heated for 20 mins, cooled and filtered.

- 2 drops of Wagner reagent were added to 1 ml of filtrate and observed for brown precipitate.
- 2 drops of Hager Reagents were added to 1 ml of the filtrate and observed for a yellow precipitate.
- 2 drops of Dragendorff reagents was added to 2 ml of filtrate and observed for brown precipitate.
- 2 drops of Mayer reagent was added to 2 ml of filtrate and observed for a milky Precipitate.

Test for saponins: Three (3) ml of distilled water was added to 2 ml of filtrate in a test tube and was shaken vigorously for 2 minutes and observed for persistent foaming (Waterman, 1993).

Ferric chloride test: To 2 ml of filtrate was added 3 ml of distilled water followed by 2 drops of 5% ferric chloride solution and observed for the formation of intense coloration.

Alkaline reagent test: To 2 ml of the filtrate was added few drops of 20% sodium hydroxide solution followed by a few drops of dilute hydrochloric acid solution and observed for the formation of intense yellow precipitate dissolve on addition of dilute acid.

Lead acetate test: To 2 ml of filtrate was added few drops of lead acetate solution and observed for the formation of milky precipitate.

Test for carbohydrates

Fehling's test: One milliliter (1 mL) of Fehling A and Fehling B reagents were mixed together, and 2 ml of it was added to one gram of crude extract and gently boiled. A red precipitate at the bottom of the test tube indicated the presence of reducing sugars.

Iodine test: One gram (1 g) of crude extract was mixed with 2 ml of iodine solution. A dark blue or purple colouration indicated the presence of carbohydrate.

Test for phytosterol: One gram (1 g) of the extract was refluxed with a solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer will evaporate and the residue tested for the presence of phytosterol by dissolving it in few drops of diluted acetic acid; 3 ml of acetic anhydride were added followed by few drops of concentrated H_2SO_4. The appearance of bluish-green colour showed the presence of phytosterol.

Test for triterpenoids: Ten milligrams (10 mg) of the extract was dissolved in 1 mL of chloroform. 1 mL of acetic anhydride was added followed by addition of 2 mL of concentrated H_2SO_4. Formation of reddish-violet colour indicates the presence of triterpenoids.

Test for phlobatannins: One gram (1 g) of extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Test for flavonoids: To 4 mL of extract, 2 mL of 10 % NaOH was added and observed for yellow colouration.

Test for terpenoids (Salkowski test): 5 mL of each extract was mixed in 2 mL of chloroform and 3 mL of concentrated H_2SO_4 was carefully added down the side of the inner wall of test tube to form a layer. A reddish brown colouration of the inter-phase is required for the presence of terpenoids.

Test for glycosides: 1ml of the extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution. This was under-layered with 1ml of concentrated H_2SO_4. A brown ring is required for the presence of glycoside.

Test for steroids: 2 mL of acetic anhydride was added to 0.5 g plant extract in 2 mL dilute H_2SO_4. A colour change from violet to blue or green is required for the presence of steroids.

Test for tannins: To 2 mL of the extract, 10 mL of distilled water was added and boiled for 5 min and then filtered into halves. To about 2 drops of the filtrate, ferric chloride ($FeCl_3$) solution was added; the formation of a bluish precipitate is required for hydrolysable tannin. To about 5 drops of the filtrate, 2 mL dilute HCl was added and boiled for 5 min. Red precipitate is required for condensed tannin.

Test for anthraquinone: 0.1 g of sample was weighed into conical flask and 10 mL of hot water was added. It was allowed to cool and extracted with 10 mL of benzene. 10 Ml of 5 % ferric chloride and 5 mL of hydrochloric acid was added to the water fraction of the extract and refluxed for 10 min. 10 mL of benzene was used to extract again. The residue was evaporated and dissolved in 10 mL of 5 % potassium hydroxide. Absorbance concentration of the solution was measure at 515 nm using anthraquinone glycoside as reference standard.

Preparation of ascorbic acid solution: 25 mL ascorbic acid solution of 250 ug/mL concentration was prepared by accurately weighing 0.0063 g of ascorbic acid, using analytical balance, into a clean dry 25 ml beaker. 10 mL of distilled water was added and the mixture was stirred with the aid of a clean glass rod until all the ascorbic acid dissolved. Thereafter, the solution carefully transferred into a clean 25 mL standard flask which was previously rinsed with distilled water. The beaker and the glass rod were then rinsed into the flask three times using about 5 mL of distilled water for each. The solution was made up with distilled water to the 25 mL mark on the standard flask. The flask was corked and content properly mixed by turning the flask up and down with one hand at its base and the other on top of the cork.

Preparation of DPPH solution: The free radical scavenging activities of each of the seed extracts were assayed using a stable DPPH standard method with little modification using the method of Baliyan *et al*. (2022) and Aini *et al*. (2019).
Measurement of free radical scavenging activities: The free radical scavenging activities of each of the seed extracts were assayed using a stable DPPH standard method of Aini *et al*. (2019) with slight modification. This was done by preparing a reaction mixture for each sample extract solution/standard solution. The reaction mixtures, control and blank were allowed to incubate in the dark for 30 min. The absorbance of the reaction mixtures was measured using UV/visible spectrophotometer at 518 nm wavelength, and the ability of the extracts to scavenge DPPH radical were calculated by following the equation:

$$\% \text{ free radical scavenging activity} = \frac{\text{Absorbance of control - Absorbance of sample/standards}}{\text{Absorbance of control}} \times 100$$

Four plants ***(****Harungana madagascariensis, Jatropha curcas, Aframomum melegueta* and *Plumbago zeylanica)* are commonly used as chewing sticks for the control of oral pathogens in Esan West Local Government**.** Other plants surveyed could not be verified, identified or authenticated and no literature to support the little indigenous information from the Natives.
Statistical analysis: Analysis of variance (ANOVA) and Dunnet's method were employed for data evaluation; $p<0.05$ was taken as statistically significant. The software package (SPSS v16) was used for data analysis.

Results

The list of the plants collected from different villages in Esan West Local Government Area for the management of oral pathogens, comprising their local names and their uses is presented in Table 1
.

Table 1: List of the plants collected from different Villages in the Esan West Local Government Area for management of oral pathogens, comprising their local names and their uses.

Plant name	Local name in Esan	Part used	Uses	Picture of plant
Harungana madagascariensis	Uruarua	Roots	Cook the root together with potash and *Xyplopia ethiopica* (Unie) until the water turns brown. Use the water to goggle the mouth against toothache (Iruekpen)	
Jatropha curcas	Ukpowaoo	Leaves	Cook the leaves. The water that comes from the cooked leaves is used to wash coated tongue in children (Ekpoma).	
Aframomum melegueta	Ohie	Seeds	Chew the seeds until the mouth become peppery, use tongue to shift the seeds and chew to the affected teeth (Okpoji).	
Plumbago zeylanica	Erhamaboekpa	Leaves	Smear the leaves on fire for 5-10 mins pound and apply to the affected teeth. (Ileh).	

Table 2 shows the qualitative phytochemical analysis of medicinal plants used in Esan West Local Government Area of Edo State. Bioactive substances such as alkaloids, glycosides, saponins, phytosterols, triterpenoids,

phlobatannins, phenols and flavonoids were found present in aqueous extract of *Harungana madagascariensis, Jatropha, curcas, Aframomum melegueta* and *Plumbago zeylanica* plants. Except carbohydrates which was absent in *Plumbago zeylanica*, steroid was absent in *Harungana madagascariensis, Jatropha curcas* and *Plumbago zeylanica*.
Terpenoids was absent in *Aframomum melegueta,* Resins was absent in *Aframomum melegueta* and *Plumbago zeylanica.* Tannins was absent in *Plumbago zeylanica,* while anthraquinones was absent in *Jatropha curcas*.

Table 2: Qualitative phytochemical analysis of medicinal plants used for oral pathogens in Esan West Local Government Area of Edo State

Phytochemicals	***Harungana madagascariensis***	***Jatropha curcas***	***Aframomum melegueta***	***Plumbago zeylanica***
Alkaloids	++	++	+	++
Carbohydrates	++	+	+	-
Glycosides	++	++	++	++
Steroid	-	-	++	-
Terpenoids	+	+	-	+
Saponins	++	++	+	++
Phytosterols	+	+	++	+
Triterpenoids	+	+	+	+
Phlobatannins	+	+	+	+
Resins	++	+	-	-
Phenols	+	+	++	+
Tannins	+	++	++	-
Flavonoids	++	++	++	++
Anthraquinones	+	-	+	++

Note: - shows absence while ++, + shows the degree of presence

Table 3 shows the quantitative phytochemical analysis of aqueous extract of *Harungana madagascariensis, Jatropha curcas, Aframomum melegueta* and *Plumbago zeylanica* plants used in Esan West Local Government Area of Edo State. Bioactive substance determined ranged as follows: Alkaloids (1.8±0.31 – 10.0±0.48), Carbohydrates (0±0.00 - 9.2±0.62), Glycosides (6.9±0.31 - 8.6±0.72), Steroid (0±0.00 - 4.8±0.40), Terpenoids (0±0.00 - 4.2±0.66), Saponins (1.2±0.50 - 8.6±0.51), Phytosterols (2.2±0.48 - 5.7±0.19), Triterpenoids (1.8±0.14 - 4.2±0.23), Phlobatannins (2.5±0.08 - 4.5±0.78), Resins (0±0.00 - 8.9±0.60), Phenols (2.1±0.57 - 6.8±0.02), Tannins (0±0.00 - 7.6±0.13), Flavonoids (5.9±0.41 - 7.9±0.48) and Anthraquinones (0±0.00 - 8.3±0.38 %). *Harungana madagascariensis* had the highest value for alkaloids, carbohydrates, glycosides, triterpenoids, phlobatannins, resins and flavonoids while *Aframomum melegueta* had the highest value for steroid, phytosterols, phenols and tannins respectively.

Table 3: Quantitative phytochemical analysis of medicinal plants used for oral pathogens in Esan West Local Government Area of Edo State

Phytochemicals (%)	***Harungana madagascariensis***	***Jatropha curcas***	***Aframomum melegueta***	***Plumbago zeylanica***
Alkaloids	10.0±0.48	9.6±0.86	1.8±0.31	8.4±0.04
Carbohydrates	9.2±0.62	3.2±0.24	2.4±0.73	0±0.00
Glycosides	8.6±0.72	8.1±0.50	6.9±0.31	7.2±0.56
Steroid	0±0.00	0±0.00	4.8±0.40	0±0.00
Terpenoids	2.6±0.44	4.2±0.66	0±0.00	2.3±0.04
Saponins	7.8±0.84	8.6±0.51	1.2±0.50	6.8±0.31
Phytosterols	3.1±0.33	2.2±0.48	5.7±0.19	3.2±0.49
Triterpenoids	4.2±0.23	3.6±0.49	3.4±0.35	1.8±0.14
Phlobatannins	4.5±0.78	3.1±0.93	2.6±0.68	2.5±0.08
Resins	8.9±0.60	2.9±0.39	0±0.00	0±0.00
Phenols	2.1±0.57	4.3±0.38	6.8±0.02	2.3±0.89
Tannins	1.5±0.27	6.5±0.53	7.6±0.13	0±0.00
Flavonoids	7.9±0.48	7.4±0.39	5.9±0.41	7.1±0.52
Anthraquinones	2.9±0.48	0±0.00	2.3±0.22	8.3±0.38

Values are expressed as mean±SEM, n=3.

Table 4 shows the antioxidant activity of aqueous extract of *Harungana madagascariensis, Jatropha curcas, Aframomum melegueta* and *Plumbago Zeylanica* plants used in Esan West, Local Government Area of Edo

State against ascorbic acid and 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH). At 1000 µg/ml, the antioxidant activity of the medicinal plants used ranged from 40.1±3.29 - 45.5±3.10, compared to ascorbic acid (62.4±3.61) and 2, 2-Diphenyl-1-Picrylhydrazyl (50.4±3.44). At 500 µg/ml, the antioxidant activity of the medicinal plants used range from 35.4±2.70 - 37.9±2.52, compared to ascorbic acid (50.4±3.43) and 2, 2-Diphenyl-1-Picrylhydrazyl (46.3±3.52). At 250 µg/ml, the antioxidant activity of the medicinal plants used range from 22.8±2.35 - 29.5±2.36, compared to ascorbic acid (46.4±3.28) and 2, 2-Diphenyl-1-Picrylhydrazyl (37.9±2.76). At 125 µg/ml, the antioxidant activity of the medicinal plants used range from 12.4±1.31 - 18.1±1.80, compared to ascorbic acid (32.9±2.33) and 2, 2-Diphenyl-1-Picrylhydrazyl (29.3±2.63). At 62.5 µg/ml, the antioxidant activity of the medicinal plants used range from 5.2±0.48 - 9.4±0.78, compared to ascorbic acid (24.6±2.40) and 2, 2-Diphenyl-1-Picrylhydrazyl (20.8±2.04). At 31.25 µg/ml, the antioxidant activity of the medicinal plants used range from 1.0±0.05 - 4.5±0.73, compared to ascorbic acid (18.9±1.72) and 2, 2-Diphenyl-1-Picrylhydrazyl (12.1±1.12) respectively.

Table 4: Antioxidant activity of medicinal plants used for oral pathogens in Esan West, Local Government Area of Edo State.

	Inhibitory activity					
Concentration (µg/ml)	***Harungana madagascariensis***	***Jatropha curcas***	***Aframomum melegueta***	***Plumbago zeylanica***	**Ascorbic acid**	**2,2-Diphenyl-1-Picrylhydrazyl (DPPH)**
1000	45.5±3.10	44.4±3.51	40.1±3.29	41.8±3.64	62.4±3.61	50.4±3.44
500	35.4±2.70	37.1±2.68	35.8±2.31	37.9±2.52	50.4±3.43	46.3±3.52
250	25.5±2.20	29.5±2.36	24.2±2.27	22.8±2.35	46.4±3.28	37.9±2.76
125	12.4±1.31	12.7±1.22	15.1±1.45	18.1±1.80	32.9±2.33	29.3±2.63
62.5	5.2±0.66	5.2±0.48	9.4±0.78	8.4±0.63	24.6±2.40	20.8±2.04
31.25	4.5±0.73	3.6±0.60	1.7±0.10	1.0±0.05	18.9±1.72	12.1±1.12
15.625	0±0.00	0±0.00	0±0.00	0.±0.00	10.9±0.89	6.2±0.43

Values are expressed as mean±SEM, n=3

Table 5 shows the antimicrobial activities of aqueous extract of *Harungana madagascariensis, Jatropha curcas, Aframomum melegueta* and *Plumbago zeylanica* plants used in Esan West, Local Government Area of Edo State against selected microorganisms. At a concentration of 100mg/ml the inhibitory effect of the tested plants against *E. coil* ranged from 12.30±2.51 - 12.40±2.41, *S. aureus* (12.05±2.76 - 12.30±2.48), *Streptococcus* sp. (12.00±2.29 - 12.15±2.47), *P. aeruginosa* (12.02±2.46 - 12.33±2.29), *P. mirabilis* (12.07±2.53 - 12.33±2.31) and *K. pneumonia* (12.00±2.39 - 12.22±2.61). 100 mg/ml used of tested plant extracts could not inhibit the growth (0±0.00 mm) on fungi species such as *A. niger* and *C. albicans.* Also *Plumbago zeylanica* extract had the highest inhibitory effect on *E. coil, S. aureus, P. aeruginosa, P. mirabilis* and *K. pneumonia.* While *Harungana madagascariensis* extract had the highest inhibitory effect on *Streptococcus* sp. respectively.

Table 5: Antimicrobial activities of medicinal plants used for oral pathogens in Esan West Local Government Area of Edo State.

	Treatment (100mg/ml)				
Isolates	***Harungana madagascariensis***	***Jatropha curcas***	***Aframomum melegueta***	***Plumbago zeylanica***	**Control (Water)**
E. coil	12.33±2.38	12.30±2.51	12.37±2.62	12.40±2.41	0±0.00
S. aureus	12.10±2.63	12.07±2.44	12.05±2.76	12.30±2.48	0±0.00
Streptococcus sp.	12.15±2.47	12.00±2.29	12.00±2.60	12.00±2.52	0±0.00
P. aeruginosa	12.02±2.46	12.07±2.72	12.20±2.81	12.33±2.29	0±0.00
P. mirabilis	12.07±2.53	12.20±2.42	12.20±2.70	12.33±2.31	0±0.00
K. pneumonia	12.05±2.57	12.00±2.48	12.00±2.39	12.22±2.61	0±0.00

Values are expressed as mean±SEM, n=3.

Table 6 shows the antimicrobial activities of aqueous extract of *Harungana madagascariensis, Jatropha curcas, Aframomum melegueta* and *Plumbago zeylanica* plants used in Esan West, Local Government Area of Edo State against selected microorganisms. At a concentration of 80mg/ml the inhibitory effect of the tested plants against *E. coil* ranged from 10.30±1.37 - 10.38±1.27, *S. aureus* (10.05±1.54 - 10.18±1.74), *Streptococcus* sp. (10.06±1.72 - 10.15±1.83), *P. aeruginosa* (10.10±1.35 - 10.50±1.63), *P. mirabilis* (10.00±1.89 - 10.24±1.64) and *K. pneumonia* (10.05±1.76 - 10.20±1.42). 80 mg/ml used of tested plant extracts could not inhibit the growth (0±0.00 mm) on fungi species such as *A. niger* and *C. albicans.* Also *Plumbago zeylanica* extract had the highest inhibitory effect on *Streptococcus* sp. and *P. aeruginosa.* While *Jatropha curcas* extract had the highest inhibitory effect on *E. coil* and *P. mirabilis* respectively.

Table 6: Antimicrobial activities of medicinal plants used for oral pathogens in Esan West, Local Government Area of Edo State.

Isolates	Treatment (80mg/ml)				
	Harungana madagascariensis	***Jatropha curcas***	***Aframomum melegueta***	***Plumbago zeylanica***	**Control (Water)**
E. coil	10.30±1.46	10.38±1.27	10.30±1.62	10.30±1.37	0±0.00
S. aureus	10.18±1.74	10.13±1.69	10.10±1.41	10.05±1.54	0±0.00
Streptococcus sp.	10.10±1.38	10.06±1.72	10.12±1.75	10.15±1.83	0±0.00
P. aeruginosa	10.10±1.35	10.19±1.41	10.50±1.63	10.25±1.36	0±0.00
P. mirabilis	10.16±1.47	10.24±1.64	10.00±1.89	10.20±1.28	0±0.00
K. pneumonia	10.15±1.37	10.05±1.76	10.20±1.42	10.12±1.67	0±0.00

Values are expressed as mean±SEM, n=3.

Table 7 shows the antimicrobial activities of aqueous extract of *Harungana madagascariensis, Jatropha curcas, Aframomum melegueta* and *Plumbago zeylanica* plants used in Esan West, Local Government Area of Edo State against selected microorganisms. At a concentration of 60mg/ml the inhibitory effect of the tested plants against *E. coil* ranged from 8.00±0.38 - 8.00±0.83, *S. aureus* (8.00±0.39 - 8.10±0.48), *Streptococcus* sp. (8.00±0.41 - 8.00±0.72), *P. aeruginosa* (8.00±0.44 - 8.10±0.71), *P. mirabilis* (8.00±0.26 - 8.00±0.83) and *K. pneumonia* (8.00±0.11 - 8.10±0.43). 60 mg/ml used of tested plant extracts could not inhibit the growth (0±0.00 mm) on fungi species such as *A. niger* and *C. albicans.* Also *Harungana madagascariensis* extract had the highest inhibitory effect on *Streptococcus* sp. and *S. aureus.* While *Jatropha curcas* extract had the highest inhibitory effect on *P. mirabilis* respectively.

Table 7: Antimicrobial activities of medicinal plants used for oral pathogens in Esan West, Local Government Area of Edo State.

Isolates	Treatment (60 mg/ml)				
	Harungana madagascariensis	***Jatropha curcas***	***Aframomum melegueta***	***Plumbago zeylanica***	**Control (Water)**
E. coil	8.00±0.52	8.00±0.38	8.00±0.83	8.00±0.62	0±0.00
S. aureus	8.10±0.48	8.00±0.39	8.00±0.67	8.05±0.49	0±0.00
Streptococcus sp.	8.00±0.72	8.00±0.41	8.00±0.58	8.00±0.36	0±0.00
P. aeruginosa	8.00±0.44	8.10±0.71	8.00±0.82	8.00±0.50	0±0.00
P. mirabilis	8.00±0.60	8.00±0.83	8.00±0.26	8.00±0.38	0±0.00
K. pneumonia	8.00±0.28	8.00±0.31	8.00±0.11	8.10±0.43	0±0.00
A. niger	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
C. albicans.	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

Values are expressed as mean±SEM, n=3.

Discussion

A significant portion of Nigeria people depends on agriculture and animal husbandry for survival. They rely mostly on the plant and animal resources of forests, wetlands, cultivated lands, and common lands (Croteau *et al.*, 2000). These resources are harvested and used in many ways, for example, as food plants, fodder plants, wild vegetables, spices/ condiments, and fruits and also for constructing huts and houses, buildings, animal sheds, as wild genetic resources for improving crop plants (Ducimetière *et al.*, 2000). People obtain important medicines from plants to maintain their health. This knowledge of the utilization of biological resources is still survived in the minds of economically poor people living in remote rural areas, nearby forests or wetlands, etc.
From the results, the phytochemical substance present in aqueous extract of medicinal plants surveyed from five different Local Government Area in Edo State shows that plants such as *Harungana madagascariensis, Jatropha curcas, Aframomum melegueta* and *Plumbago zeylanica* contained bioactive substances such as alkaloids, carbohydrates, glycosides, steroid, terpenoids, saponins, phytosterols, triterpenoids, phlobatannins, resins, phenols, tannins, flavonoids and anthraquinones in different proportions. This was in line with work of Duster and Waters (2006) who reported the presence of some phytochemical compound in some plant extracts used in Benin City. (Dybas, 2007; NCADR, 2017) have also reported that the ability of plant extracts to inhibit the growth of microorganisms is attributed to the presence of phytochemicals inherent in the plant and responsible for it broad-spectrum antimicrobial activities. Phytochemicals have been identified by different researchers and have used from prehistoric times. These chemical compounds perform several biological

functions and they work on the human body in exactly the same way as pharmaceutical drugs (Manjuola *et al*., 2019).

Edgar *et al.* (2002; Pilarska *et al*., 2017) also reported that these bioactive substances are naturally occurring compounds in plant and they have been known to provide various biological functions in humans. In particular, carotenoids, flavonoids, terpenoids, saponins, phytosterols, triterpenoids, phlobatannins and phenols are the most extensively studied phytochemicals for their antioxidant functions as well as potential preventive medicinal benefits such as maintaining inflammation balance, reducing the risk of certain cancers, and promoting cardiovascular, neurocognitive, eye, and bone health in humans (Egede *et al.,* 2002).

Quantitative phytochemical analysis of these medicinal plants used in the Local Government surveyed also shows that alkaloids (1.8±0.31 – 10.0±0.48), carbohydrates (0±0.00 - 9.2±0.62), glycosides (6.9±0.31 - 8.6±0.72), steroid (0±0.00 - 4.8±0.40), terpenoids (0±0.00 - 4.2±0.66), saponins (1.2±0.50 - 8.6±0.51), phytosterols (2.2±0.48 - 5.7±0.19), triterpenoids (1.8±0.14 - 4.2±0.23), phlobatannins (2.5±0.08 - 4.5±0.78), resins (0±0.00 - 8.9±0.60), phenols (2.1±0.57 - 6.8±0.02), tannins (0±0.00 - 7.6±0.13), flavonoids (5.9±0.41 - 7.9±0.48) and anthraquinones (0±0.00 - 8.3±0.38) were seen to be present in various quantities. These results is in line with the work of Ellof (2008) who reported that these bioactive substances are naturally occurring compounds in different plant extracts to provide various biological functions in humans. For example, alkaloids can act as antimalarial, anticancer, antiasthma and antibacterial pharmacological constituents in humans. Tannins on the other hand have been used to combat diarrhea (Elvin-Lewis, 2001). The presence of tannins also enhances the antioxidant properties of the different medicinal plants studied (Jansen *et al.,* 2010). Saponins have gained grounds as a dietary supplement and nutraceutical (Jensen *et al.,* 2014). Plant saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccines and knock out some kinds of tumor cells particularly lung and blood cancers (Archana and Gupta, 2020).. They also lower blood cholesterol thereby reducing heart disease. The most outstanding and exciting prospect for saponins is how they inhibit or kill cancer cells. They may also be able to do it without killing normal cells, as is the mode of some fighting drugs. Cancer cells have more cholesterol-type compounds on their membranes than normal cells. Saponins therefore bind cholesterol and thus interfere with cell growth and division (Kala and Prakash, 2007).

The antioxidant activity of different plant extracts at varied concentration shows that they were all significantly rich in antioxidant. The antioxidant activity of the different plant extracts used at 1000 µg/ml ranged from 40.1±3.29 - 45.5±3.10, compared to ascorbic acid (62.4±3.61) and 2, 2-Diphenyl-1-Picrylhydrazyl (50.4±3.44). At 500 µg/ml, the value range from 35.4±2.70 - 37.9±2.52, compared to ascorbic acid (50.4±3.43) and 2, 2-Diphenyl-1-Picrylhydrazyl (46.3±3.52). At 250 µg/ml, the value range from 22.8±2.35 - 29.5±2.36, compared to ascorbic acid (46.4±3.28) and 2, 2-Diphenyl-1-Picrylhydrazyl (37.9±2.76). At 125 µg/ml, the value range from 12.4±1.31 - 18.1±1.80, compared to ascorbic acid (32.9±2.33) and 2, 2-Diphenyl-1-Picrylhydrazyl (29.3±2.63). At 62.5 µg/ml, the value ranged from 5.2±0.48 - 9.4±0.78, compared to ascorbic acid (24.6±2.40) and 2, 2-Diphenyl-1-Picrylhydrazyl (20.8±2.04). At 31.25 µg/ml, the value ranged from 1.0±0.05 - 4.5±0.73, compared to ascorbic acid (18.9±1.72) and 2, 2-Diphenyl-1-Picrylhydrazyl (12.1±1.12) respectively. This was also in accordance with the work of Ivanišová *et al.* (2013), who reported that the antioxidant activity of five medicinal plant extracts was 10.453 - 9.832 compared to Butylated hydroxytoluene (BHT), Ascorbic acid and α-Tocopherol, which was 12.981, 22.615 and 11.465. Izzo *et al.* (2005) reported that antioxidants compound present in plant are natural substances that may prevent or delay some types of cell damage, they have also been reported to be found in many foods, including fruits and vegetables (Idu *et al.,* 2007). The significant range of antioxidant activities present in the different plant extracts used in this study, compared to already well-established antioxidant chemicals, indicates that all the different plant extracts can be used in traditional and modern medicine and further developed into pharmaceutical products (Agbabiaka *et al.,* 2017).

In addition Kathad *et al.* (2010) reported that antioxidants are compounds that inhibit oxidation, and that oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions (Khanna and Tosh, 2014). To balance the oxidative state, human and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (catalase and superoxide dismutase), produced internally, or the dietary antioxidants like vitamin C, and vitamin E which have been shown to improve health in humans by the preventing disease. More so Klonoff (2016) reported that antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (lipophilic). Water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation. These compounds may be synthesized in the body or obtained from the diet (Adaramola *et al.,* 2012).

The results of antimicrobial activities of different concentrations extracts of the plants used in the present study reveal that all different plant concentrations extracts are potent antimicrobials against all the pathogenic organisms studied. The antibacterial activity was screened from the zone of inhibition. Among the various concentrations (100, 80 and 60 mg/ml) of extracts studied, 100 mg/ml showed the higher degree of inhibition on

E. coil (11.00±2.55 - 13.00±2.26), *S. aureus* (10.50±2.31 - 13.00±2.42), *Streptococcus* sp. (10.50±2.62 - 12.00±2.23), *P. aeruginosa* (10.02±2.60 - 12.05±2.73), *P. mirabilis* (11.00±2.32 - 12.00±2.66) and *K. pneumonia* (10.40±2.65 - 13.10±2.81). Followed by 80 mg/ml, on *E. coil* (10.01±1.05 - 11.10±1.21), *S. aureus* (10.00±1.08 - 11.52±1.28), *Streptococcus* sp. (10.01±1.20 - 11.48±1.23), *P. aeruginosa* (10.07±1.23 - 11.36±1.14), *P. mirabilis* (10.10±1.22 - 11.19±1.19) and *K. pneumonia* (10.00±1.18 - 11.50±1.20) respectively. The aqueous extracts of the different plants showed no minimum inhibitory effect on any of the fungi species tested. A similar result was observed in the study of (Amrulloh and Fatiqin, 2020) using ethanol and water extracts of the *I. trichantha* leaves against tested microorganisms. Their results also showed that bacteria organisms tested were more susceptible to the 100 mg/ml than 60 mg/ml concentration. The antimicrobial activities of the different plants used in the present study have been reported by many researchers (Almagboul *et al.*, 2005; Idu *et al*., 2007). The emergence of antibiotic resistance has its roots in the injudicious use of antibiotics and the subsequent transfer of resistance genes and bacteria among animals, animal products and environment. Extra chromosomal genes associated with plasmids were responsible for these antibacterial resistant phenotypes that may impact resistance to an entire antibacterial class (Amorati and Valgimigli, 2012). Thus as the plant produce secondary metabolites in order to protect themselves from microorganisms, herbivores and insects, thus the antimicrobial effect is somehow expected from plants, namely flavonoids, alkaloids and triterpenoid and are producing a better opportunity for testing a wide range of microorganism.

Conclusion

Illness and infections are often caused by the growth of many pathogenic bacterial strains. Prevention of these pathogenic microorganisms is mainly based on the application of chemical drugs. The adverse effect of these chemical substances on human health increases the demand to search for potentially effective, healthy, safer and natural bactericide. *Harungana madagascariensis*, *Jatropha curcas*, *Aframomum melegueta* and *Plumbago zeylanica* plant extracts have proved to be potentially effective on *E. coil*, *S. aureus*, *Streptococcus* sp., *P. aeruginosa*, *P. mirabilis* and *K. pneumonia*. Due to the presences of phytochemicals such as alkaloids, carbohydrates, glycosides, steroid, terpenoids, saponins, phytosterols, triterpenoids, phlobatannins, resins, phenols, tannins, flavonoids and anthraquinones inherent in the plants. The study suggests the used as natural alternative to treat diseases and to preserve human lives.

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