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Phytochemical screening and antimicrobial properties of partially purified ethyl acetate extracts of *Erythrina senegalensis* leaf and bark against selected clinical isolates

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Authors' contributions

This work was carried out in collaboration among all authors. Author **OOT** is the sole author who designed, analyzed, interpreted and prepared the manuscript for publication. Author OOT is a researcher and has researched into the antimicrobial and phytochemical properties of various medicinal plants in Nigerian and Africa. Author **AAO** helps in proof reading the entire manuscript in preparation for publication and also helps during the antimicrobial assays. Author **OTA** helps to proof read the first and the final manuscript before publication. Author **TOA and TOV** helps during the chromatograph procedure and the chemical analysis of the plant extracts.

Abstract
Erythrina senegalensis DC. (Coral tree) is commonly grown in West Africa as an ornamental plant and one of the oldest known African medicinal plants. The purpose of this research is to determine the antimicrobial potency, phytochemical activities, nutrients and elemental composition of the leaf and bark of *Erythrina senegalensis*. The organisms used for the study are *Escherichia coli, staphylococcus aureus, Candida albicans and Klebsiella pneumonia*. The leaf and bark were extracted using one extracting solvent (ethyl acetate) and three extracting solvent (N-hexane, ethyl acetate and ethanol) for elution. *Erythrina senegalensis* extracts were partially purified using the column chromatography method. The antimicrobial potency of the partially purified extracts of *Erythrina senegelensis* was determined using the agar dilution method. Quantitative and quantitative phytochemical screening of *Erythrina senegelensis* were also observed and recorded accordingly. The antimicrobial activity of *Erythrina senegalensis* extracts of both leaf and bark, shows the antimicrobial sensitivity test of *Erythrina senegalensis* against the test organisms. Fraction one (F1) and fraction (F2) of the *Erythrina senegalensis* bark extracts are more effective on *Candida albican* and *Staphylococcus aureus* were susceptible while the fraction two (F2) and fraction (F3) of the leaf extract are lesser in antimicrobial activity. The qualitative and quantitative phytochemical screening of *Erythrina senegalensis* shows that the *Erythrina senegalensis* contains Saponins, Flavonoids, Phylate, Alkaloids, Phenol, Tannin and Oxalate. *Erythrina senegalensis* also contains minerals such as Sodium(Na), Calcium(Ca), Potassium(K), Magnesium(Mg), Zinc(Zn), Iron(Fe), Copper(Cu) and Manganese (Mn). Nutrient compositions of the *Erythrina senegalensis* are also of great importance to human survival. As a result of the effectiveness of *Erythrina senegalensis* extracts on the test organisms used, it is consider as potential source of antimicrobial agents against life threatening diseases.

Keywords: Antimicrobial activity, Purified fraction, phytochemical activity, proximate composition, elemental constituent.

1. Introduction

Human beings have used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982)[48]; Hill, 1989) [11]. According to the World Health Organization, most

populations still rely on traditional medicines for their psychological and physical health requirements (Rabe Stoden, 2000) [35], since they cannot afford the products of Western pharmaceutical industries (Salie *et al.*, 1996) [36], together with their side effects and lack of healthcare facilities (Griggs *et al*., 2001) [9].

Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu *et al.*, 1999; Idu *et al*., 2007; Mann *et al*., 2008; (Musa 2009)[49] [17, 16, 22]. People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses (Maheshwari *et al.,* 1986; Van Wyk *et al.,* 2000) [21, 43].

Erythrina senegalensis DC (Fabaceae) is a thorny shrub or small tree with common names that include coral tree (English) and minjirya (Hausa, Nigeria). It is 5-15m tall and grows in West-Africa tropical and sub-tropical areas from Senegal to Cameroon. The stem and root bark are used by traditional healers to cure wide range of illnesses (Adamu *et al*., 2005)[50]; Togola *et al.,* 2008; Kone *et al.,* 2011) [40, 18]. The leaves are used to treat malaria, gastrointestinal disorders, fever, dizziness, secondary sterility, diarrhea, jaundice, nose bleeding and pain (Togola *et al.,* 2008) [40].

The stem bark extract has been shown to have antimicrobial activity against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa*, *Aspergillus flavus, Aspergillus fumigatus*, *Candida albicans, Penicillium notatum* (Doughari, 2010) [51] and inhibitory activity against HIV-1 protease (Lee *et al.,* 2009) [19]. The root ethanol extract exhibited strong activity against *Plasmodium falciparum*. The stem bark has been shown to have hepatoprotective properties (De *et al*,.2002)

Phytochemicals such as tannins, glycosides, alkaloids, cardiac glycosides, prenylated isoflavones and flavones have also been identified in the *Erythrina senegalensis* stem bark (Wandji *et al.,* 1994; Oh *et al.,* 1999; [45, 46]. The leaf has also been shown to contain many phytochemicals (Bako and Madu, 2007). Although appreciable data has been reported on the stem and root bark, little is known about the leaf extract. In this study, we evaluated the effect of *Erythrina senegalensis* aqueous leaf extract orally administered in rats.

2. Materials and Methods

Collection and Preparation of Plant Leaf and Bark Extract

The fresh leaf and bark samples of study the plant *Erythrina senegalensis* was collected from St Catherine Anglican girls' college Owo in Ondo State, Nigeria on the 30th of October 2015 at around 3:45pm with a cutlass.

2.1:1 Preparation of Extract

The materials were washed and dried at room temperature and then reduced to coarse powder by slicing and grinding into almost powdery form. A 400g of dried grinded leaves and bark was separately extracted with ethyl acetate solvent of 1200ml each. This is done in a rubber bottle. Each bottle is labeled as regard what it contains. At ratio 1:3.The mixtures are allowed to soak for about 9 days. The mixture was shaken thoroughly throughout these nine days of soaking. It is kept in a cool dry place. After the 9th day, the extracts are filtered out using the separating funnel and the filter paper. Extracts of each mixture are collected into conical flask and labeled properly. (Ugbogu *et al*., 2000), (Osuntokun *et aL*,.2016)[A] [42, 33]

2.1:2 Sterilization

An autoclave was used for sterilization of various glass wares and most especially the media. Slants were made for the collection of clinical specimens (organisms). Surface of benches used were disinfected by swabbing with cotton wool soak with ethanol. All bench work and activities were done according to laboratory standards. Media were prepared in 15minutes at 121 ^{0}C.

Test Organisms

Test organisms used in the experiment were collected from Department of Microbiology of the Obafemi Awolowo University Ile Ife Osun State, Nigeria. They include: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and Candida *albicans.*

2.2 Purification of Extracts

The ethyl acetate extract is purified using the column chromatography.

2.3 Column Chromatography

Apparatus/solvents: column chromatography glass ware, silica gel, N─hexane, ethanol, ethyl acetate, cotton wool, stopcock, glass rod.

Procedure

The column is clamped vertically.

A small piece of glass or cotton wool is used as a plug to support the adsorbent, a long glass rod is used to place the wool at the bottom of the column. The wool should be compressed enough to support the column packing yet loose enough that the solvent flow will not be hindered. The desired amount of Silica gel is weighed into a conical flask, with a funnel, the wet gel is added to the column. The column is tapped gently to pack the silica gel. The desired solvent is carefully added to the top of the column and allowed to percolate through the adsorbent. The stopcock is opened and some of the solvent is allowed to drain. After the cotton wool is placed beneath the column, the silica gel is mixed with N-hexane and poured gently with the solvent (N-hexane) into the column tube. The sample is added to the top of the column after the packing of the silica gel is complete. N-hexane is first used to elute, followed by ethyl acetate and finally with ethanol. These are done for both leaves and bark extracts from ethyl acetate. Here different solutions are produce from each extract, that is the leaf and bark extracts. They are labeled respectively and according to their extract. Time of elute are also noted.

Fig 1: Using the column chromatography to elute the bark and leaf extracts of *Erythrina senegalensis* and the reagents used.

2.3:1 Test for the antimicrobial activity and minimum inhibitory concentration of leaf and bark

The antimicrobial activities of the purified extracts were evaluated by Agar dilution method. After purification the extracts were used on the test organisms. The glass wares were sterilized using the ovum at 160 ^{0}C for 2 hours. Serial dilution of the extract is made using 8 concentrations (20g/mil, 10g/mil, 5g/mil, 2.5g/mil, 1.25g/mil, 0.625g/mil, 0.3125g/mil and 0.15625g/mil) of the purified extract of *Erythrina senegelensis* were prepared. 1mil or gram per dry mass after calculations using. The C1V1≡ C2v2 formula (Clinical Laboratory Science Institute) (Osuntokun 2015) [31]

That is the volume of the sterile distilled water in the first tube other tubes contain 4mil each. 4mil is pipette from the first to the second and repeated for the 8 tubes, thereby the last tube containing 8mils. After making serials of the extracts in the tubes labeled respectively, 1mil is drawn using the graduated needle and syringe and dispensed into 19mil of prepared Mueller Hilton agar in a universal bottle. Shake properly and dispense in the sterilized plates respectively. Allow to solidify, put them a sterile hot air ovum for the media containing the extract to solidify properly. (Osuntokun2015) [31].

In a sterile environment divide the plate into four compartments, inoculate the organisms using the sterile inoculating loop in each part of the divide plate, labeled appropriately. Repeat the procedure for all samples obtained from the purified extracts. Incubate at 37 ^{0}C for 16 to 20 hours inverted. The compartment in the plate with the lowest dilution with no detectable growth on the naked eyes was considered as the MIC value.

Reactivation of test organisms

The stock culture of the provided organisms were re activated by inoculating the organisms using a wire loop from the stock culture into a sterile nutrient broth and incubated for 18─24 hours (overnight incubation).

Standardization of Organisms.

The organisms is standardized by measuring out 0.1ml of fresh overnight culture of the test organisms grown in nutrient broth into 9.9ml of sterile distilled water in a test tube, 0.1 is picked again from the second tube into the third tube. From the standardized organism in the third tube is where test organism is picked for inoculation on prepared media containing the extract (Osuntokun *et al*,. 2016)^B [32].

2.3:3 Culture Media

Mueller Hinton agar was used for the preparation of plates used for the antimicrobial testing by following manufacturer instructions.

2.4 Determination of Phytochemical Screning, Elemental Composition, Anti-Nutruient and Proximate Composition of *Erythrina Senegalensis*.

2.4:1 Qualitative Method of Analyses

Preliminary test / Preparation test

Plant filtrate was prepared by boiling 20 g of the fresh plant in distilled water. The solution was filtered through a vacuum pump. The filtrate was used for the phytochemical screening or Flavonoids, tannins, Saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides. (Ahmedulla, 1999) [1].

(i) Test for Alkaloids

About 0.2gram was warmed with 2% of H_2SO_4 for two minutes, it was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicates the present of Alkaloids.

(ii) Test for Tannins

One milliliter of the filtrate were mixed with 2m1 of FeC1, A dark green colour indicated a positive test for the tannins. (Edeoga 2005) [6]

(iii) Test for Saponins

One milliliter of the plant filtrate were diluted with 2 ml of distilled water; the mixture were vigorously shaken and left to stand for 10min during which time, the development of foam on the surface of the mixture lasting for more than 10mm, indicates the presence of Saponins. (Edeoga 2005) [6]

(iv) Test for Anthraquinones- One milliliter of the plant filtrate was shaken with 10ml of benzene; the mixture was filtered and 5 ml of 10% (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a positive test (Edeoga 2005) [6]

(v) Test for Anthocyanosides

One milliliter of the plant filtrate was mixed with 5 m1 of dilute HCI; a pale pink colour indicates the positive test.

(vi) Test for Flavonoids

One milliliter of plant filtrate was mixed with 2 m1 of 10% lead acetate; a brownish precipitate indicated a positive test for the phenolic Flavonoids. While for flavonoids, I m1 of the plant filtrate were mixed with 2m1 of dilute NaOH; a golden yellow colour indicated the presence of Flavonoids. (Ekpo, 2009) [7].

(vii) Test for Reducing Sugars

One milliliter of the plant filtrate was mixed with Fehling A and Fehling B separately; a brown colour with Fehling B and a green colour with Fehling A indicate the presence of reducing sugars. (Ekpo, 2009) [7].

(viii) Test for Cyanogenic glycosides

This was carried out subjecting 0.5g of the extract 10ml sterile water filtering and adding sodium picrate to the filtrate and heated to boil. (Ekpo, 2009) [7].

(ix) Test for Cardiac glycosides

Legal test and the killer-kiliani was adopted, 0.5g of the extract were added to 2ml of acetic anhydrate plus H_2S0_4.

2.4:2 Quantitative Method of Analyses

(i) Saponins

About 20grams each of dried plant samples were ground and, put into a conical flask after which 100 ml of 20% aqueous ethanol were added. The mixture was heated using a hot water bath. At about 55 °C, for 4 hour with continuous stirring, after which the mixture were filtered and the residue re-extracted with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 rnl of diethyl ether were added and then shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times. 60 rnl of n-butanol were added. The combined n-butanol extracts were washed twice with 10 m1 of 5% aqueous sodium chloride. The remaining solutions were heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content

was calculated as percentage of the starting material. (Guevarra 2005) [10].

(ii) Flavonoids

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solutions were filtered through Whatman filter paper No 42. The filtrate were later transferred into a crucible and evaporated into dryness over a water bath; the dry content was weighed to a constant weigh. (Guevarra 2005) [10].

(iii) Cardiac glycosides

Legal test and the killer-kiliani was adopted, 0.5g of the extract were added to 2ml of acetic anhydrate plus H_2S0_4.

(iv) Tannins

About 500 mg of the plant sample were weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M Fecl in 0.1 M Hcl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract. (Mahato, 1997) [20].

(v) Alkaloids

Five grams of the plant sample were weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was then be added, the reaction mixture were covered and allowed to stand for 4 hour. These were filtered and the extract will be concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation is complete. The whole solution were allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass. (Mary, 2008) [25]

(vi) Phlobatannins

About 0.5grams of each plant extracts were dissolved in distilled water and filtered. The filtrates were boiled in 2% Hcl, red precipitate show the present of Phlobatannins. (Sofowora, 1982) [39].

Determination of Proximate Analysis of *Erythrina senegalensis*

The proximate parameters (moisture, dry matter, ash, crude fats, proteins and fibers, nitrogen, carbohydrates and energy values) were determined using Association of Official Analytical Chemists Methods. (Trease and Evans 1983) [41]

1. Determination of moisture content was done by drying samples in oven (WiseVen, WON-50, Korea) at 110 °C until constant weight was attained (Horwitz 2003) [12].
2. Nitrogen estimation was carried out by the micro-Kjeldahl (BUCHI, KjelFlex K-360, and Switzerland) method with some modification. (Hussain *et al*,.2011) [14].
3. The crude proteins were subsequently calculated by multiplying the nitrogen content by a factor of 6.25, Hussain*et al*,.2011 [14] The energy value estimation was done by summing the multiplied values for crude protein, 4 Crude fat and carbohydrate respectively at Water Factors (4, 9 and 4). Crude fats were determined by Soxhlet apparatus using *n*-hexane as a solvent.
5. The ash values were obtained by heating samples at 550 °C in a muffle furnace (Wise Them, FHP-03, Korea) for 3 h. (Hussain *et al*,.2011). [14]
6. The carbohydrate content was determined by subtracting the total crude protein, crude fiber, ash content and crude fat from the total dry matter (Horwitz, 2003) [13].
7. Crude fiber was estimated by acid-base digestion with 1.25% H_2SO_4 (v/v) and 1.25% NAOH (w/v) solutions Al-(Harrasi *et al*, .2012) [2].

Results

Table 3:2.*Candida albicans* has the highest zone of inhibition at concentrations 20(mg/ml), 10(mg/ml), and 5mg/ml) with 7.0, 5.0 and 4.0 respectively while *Klebsiella pnuemonia* has the lowest value at the same concentration. *Escherichia coli* and *Staphylococcus aureus* have the same value at concentration 20(mg/ml) while in concentration 10(mg/ml), *Escherichia coli* have a higher value than *Staphylococcus aureus*. At concentration 2.5(mg/ml), *Klebsiella pneumonia* and *Escherichia coli* have the same highest value of 2.0 and lowest value of 0.0 while *Staphylococcus aureus* and *Candida albicans* have the value of 2.0 and 1.0 at concentration 2.5(mg/ml) and 1.25(mg/ml) respectively. Only *Escherichia coli* grow at concentration 0.625(mg/ml). The MIC was at concentration 0.3125(mg/ml) and 0.1562(mg/ml).

Table3:3, at concentrations 20(mg/ml) and 10(mg/ml), *Staphylococcus aureus* have the highest zone of inhibition at 11.0 and 7.0 respectively followed by *Klebsiella pneumonia* at 9.0 and 6.0 in the same concentration. The value of *Escherichia coli* is more than *Candida albicans* at concentration 20(mg/ml) with 1.0 difference and vice versa in concentration 10(mg/ml).*Staphylococcus aureus* and *Klebsiella pneumonia* have the same highest value of 5.0 at concentration 5(mg/ml) while *Candida albicans* and *Escherichia coli* have the same lowest value of 3.0 at the same concentration. At concentrations 2.5(mg/ml) and 1.25(mg/ml), *Staphylococcus aureus* have the same highest value of 4.0 while *Klebsiella pneumonia* have the same lowest value of 2.0 at the same concentrations. *Candida albicans* and *Klebsiella pneumonia* have the same value of 3.0 at concentration 2.5(mg/ml) while the value of *Klebsiella pneumonia* is more than that of *Candida albicans* in concentration 1.25(mg/ml).*Staphylococcus aureus* has the highest value of 3.0, followed by *Klebsiella pneumonia* with 2.0 and *Candida albicans* and *Escherichia coli* having the same least value at concentration 0.625(mg/ml). *Staphylococcus aureus* shows growth at concentration 0.3125(mg/ml) while the MIC is at concentration 0.1562(mg/ml).

Table3:4, shows that *Escherichia coli* has the highest zone of inhibition of 9.0 and 7.0 at concentrations 20(mg/ml) and 10(mg/ml), followed by *klebsiella pneumonia* with 8.0 and 6.0, then *Staphylococus aureus* with 6.0 and 5.0 while *Candida albicans* has the least value of 5.0 and 3.0 at that concentrations. At concentration 5(mg/ml), *Escherichia coli* and *Staphylococcus aureus* have the same highest value of 4.0 while *Klebsiella pneumonia* is more than *Candida albicans*. At 2.5(mg/ml),*Escherichia coli* is the highest with 3.0 while *Klebsiella pneumonia* is the lowest with 1.0, *Staphylococcus aureus* and *Klebsiella pneumonia* has the same value of 2.0. At concentration 1.25(mg/ml), *Staphylococcus aureus, Candida albicans* and *Klebsiella pneumonia* have the same least value of 1.0 while *Escherichia coli* is the highest with 2.0. At concentration 0.625(mg/ml), only *Staphylococcus aureus* do not show growth while others show growth. The MIC value is at 0.3125 (mg/ml) concentrations.

Table 3;5, shows that Escherichia *coli* has the highest zone of inhibition with followed by *Staphylococcus aureus*, *Candida*

albicans and *Klebsiella pneumonia* having the least at concentration 20(mg/ml)) with7.0, 6.0 and 5.0 respectively .At concentration 10(mg/ml), *Staphylococcus aureus* has the highest value of followed by *Escherichia coli* and *Klebsiella pneumonia* having the least value with 5.0,4.0 and3.0 respectively. At concentrations 2.5(mg/ml) and 1.25(mg/ml), *Staphylococcus aureus* and *Escherichia coli* have the same value of 2.0 while *Klebsiella pneumonia* has the least value. But *Candida albicans* is greater at concentration 2.5 (mg/ml) with 2.0.*Candida albicans* has the same value as *Escherichia coli* and *Staphylococcus aureus* with 1.0. The MIC is at concentration 0.625(mg/ml).

Table 3:6, shows that *Klebsiella pneumonia* has the highest zone of inhibition at concentrations 20(mg/ml), 10(mg/ml), 5(mg/ml) and 2.5(mg/ml) with 8.0, 5.0, 4.0and 3.0 respectively while *Staphylococcus aureus* and *Escherichia coli* have the lowest value at the same concentrations with5.0, 3.0 2.0 and 1.0.*Candida albicans* has more value than *Staphylococcus aureus* and *Escherichia coli* at the same concentration. At concentration 1.25(mg/ml), *Candida albicans* and *Klebsiella pneumonia* have the highest value while *Staphylococcus aureus* and *Escherichia coli* have the lowest value. At concentration 0.625, *Candida albicans* and *Klebsiella pneumonia* have the same value of 2.0, while at concentrations 0.625(mg/ml), 0.3125(mg/ml) and 0.1562(mg/ml) only *Staphylococcus aureus* has the same value of 1.0.

Table3:7, at concentrations 20(mg/ml), 10(mg/ml) and 5(mg/ml) *Escherichia coli* has the highest zone of inhibition while *Klebsiella pneumonia* has the least value at the same concentration. *Candida albicans* has a greater value than *Staphylococcus aureus* and *Escherichia coli* at the same level of concentration. At concentration 1.25(mg/ml), *Candida albicans* and *Klebsiella pneumonia* have the highest value while *Staphylococcus aureus* and *Escherichia coli* have the lowest zone of inhibition. At concentrations 0.625(mg/ml) and 0.3125(mg/ml), *Staphylococcus aureus* and *Klebsiella pneumonia* have the same value of 1.0 while *Escherichia coli* in concentration 0.625(mg/ml) is more than that of concentration 0.3125(mg/ml) with a difference of 1.0.

Table 3:8, this shows the phytochemical screening of Erythrina senegelensis in alkaloids, cardiac glycosides, steroids, anthraquinone, phenol, tannins, and Saponins are present while Flavonoids is not present in the leaf extracts. All the elemental compositions are present in the leaf of the plant except anthraquinone which was not determined.

Table3:9, shows the mineral composition of the Erythrina senegelensis in which lead (Pb) is not determined. Sodium (Na), Potassium (K), Magnesium (Mg), Zinc (Zn), Iron (Fe), Copper (Cu) and Manganese were all determined in both bark and leaf of *Erythrina senegelensis*. Calcium has the highest value of 31.49 in bark and 32.98 in leaf of the plant while Copper has the least value of 0.01 in the bark and 0.02 in the leaf extracts.

Table3:10, shows the ant-nutrients present in the plant extracts in (%). They includes; tannin, phenol, phylate, oxalate, Flavonoids and alkaloid. All were all determined in both bark and leaf except alkaloid which was not determined in the leaf extracts. Saponin has the highest value in with 10.26% leaf with 11.23%.The least value occur in phylate and oxalate of both bark and leaf of the plant.

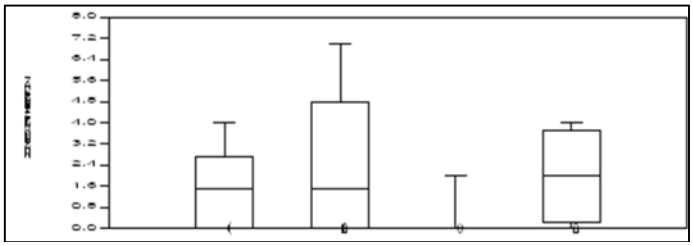
Table3:11, shows the proximate nutrient present in *Erythrina senegelensis* extracts in (%).They are: ash, crude, protein, fibre, fat, moisture content and carbohydrate. Carbohydrate has the highest value of 53.74 in bark and 50.12 in leaf. The least proximate nutrient is fat having 5.42 in barks and 5.43 in leaf.

Table 3.1: 1 Local Names, Part Used and Botanical Names

Local Name	Part Used	Botanical Names
Obobo (Leaf and Bark)	**Leaf and Bark**	***Erythrina senegalensis***

Table 3.2: Fraction one antibacterial activities of ethyl acetate bark extract using N hexane to elute.

Concentrations (mg/ml)	*Staphylococcus aureus*	*Candida albicans*	*Klebsiella pneumonia*	*Escherichia coli*
20	4.0	7.0	2.0	4.0
10	3.0	5.0	0.0	4.0
5	2.0	4.0	0.0	3.0
2.5	2.0	2.0	0.0	2.0
1.25	1.0	1.0	0.0	2.0
0.625	0.0	0.0	0.0	1.0
0.3125	0.0	0.0	0.0	0.0
0.1562	0.0	0.0	0.0	0.0



susceptible≤ 4µg/mil or 20,Antimicrobial with interpretative criteria using CLSI standard Unit of zone of inhibition (mm) susceptible≤ 4µg/mil or 20

Fig 2: Graphical representation of Fraction one antibacterial activities of ethyl acetate bark extract using N hexane to elute.

KEYS

A-*Staphylococcus aureus,* B-*Candida albicans***,** C-*Klebsiella pneumonia***,** D-*Escherichia coli*

Table 3.3: Fraction two antibacterial activity of ethyl acetate bark extract using ethyl acetate to elute.

Concentrations (mg/ml)	*Staphylococcus aureus*	*Candida albicans*	*Klebsiella pneumonia*	*Escherichia coli*
20	11.0	5.0	9.0	6.0
10	7.0	6.0	6.0	5.0
5	5.0	3.0	5.0	3.0
2.5	4.0	3.0`	3.0	2.0
1.25	4.0	2.0	3.0	2.0
0.625	3.0	1.0	2.0	1.0
0.3125	1.0	0.0	0.0	0.0
0.1562	0.0	0.0	0.0	0.0

Antimicrobial with interpretative criteria using CLSI standard. Unit of zone of inhibition (mm) susceptible ≤ 4µg/mil or 20mm

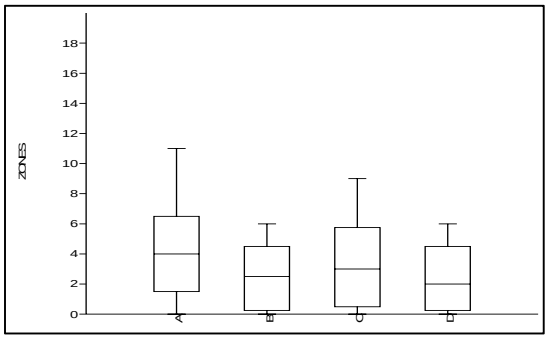


Fig 3: Graphical representation of Fraction two antibacterial activity of ethyl acetate bark extract using ethyl acetate to elute.

Table 3.4: Fraction three antibacterial activity of ethyl acetate bark extract using ethanol to elute.

Concentrations (mg/ml)	*Staphylococcus aureus*	*Candida albicans*	*Klebsiella pneumonia*	*Escherichia coli*
20	6.0	5.0	8.0	9.0
10	5.0	3.0	6.0	7.0
5	4.0	2.0	3.0	4.0
2.5	2.0	1.0	2.0	3.0
1.25	1.0	1.0	1.0	2.0
0.625	0.0	1.0	1.0	1.0
0.3125	0.0	0.0	0.0	0.0
0.1562	0.0	0.0	0.0	0.0

Antimicrobial with interpretative criteria using CLSI standard. Unit of zone of inhibition (mm) susceptible ≤ 4µg/mil or 20mm

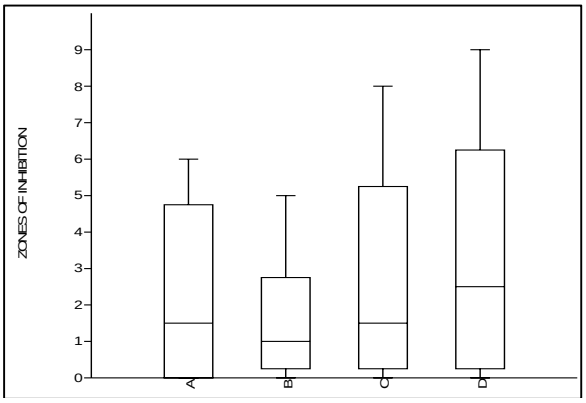


Fig 4: Graphical representation of Fraction three antibacterial activity of ethyl acetate bark extract using ethanol to elute.

Table 3.5: Fraction one antibacterial activity of ethyl acetate leaf extracts using N hexane to elute.

Concentration (mg/ml)	*Staphylococcus aureus*	*Candida albicans*	*Klebsiella pneumonia*	*Escherichia coli*
20	6.0	5.0	0.0	7.0
10	5.0	3.0	0.0	4.0
5	3.0	2.0	0.0	3.0
2.5	1.0	2.0	0.0	1.0
1.25	1.0	1.0	0.0	1.0
0.625	0.0	0.0	0.0	0.0
0.3125	0.0	0.0	0.0	0.0
0.1562	0.0	0.0	0.0	0.0

Antimicrobial with interpretative criteria using CLSI standard. Unit of zone of inhibition (mm), Susceptible ≤ 4µg/mil or 20mm

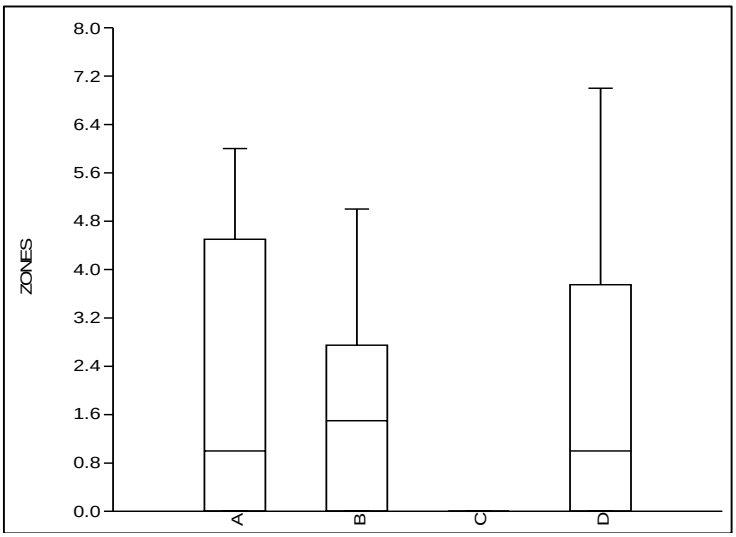


Fig 5: Graphical representation of Fraction one antibacterial activity of ethyl acetate leaf extracts using N hexane to elute.

Table 3.6: Fraction two antibacterial activities of ethyl acetate leaf extract using ethyl acetate to elute.

Concentrations (mg/ml)	*Staphylococcus aureus*	*Candida albicans*	*Klebsiella pneumonia*	*Escherichia coli*
20	5.0	6.0	8.0	5.0
10	3.0	4.0	5.0	3.0
5	2.0	3.0	4.0	2.0
2.5	1.0	2.0	3.0	1.0
1.25	1.0	2.0	2.0	1.0
0.625	1.0	2.0	2.0	0.0
0.3125	1.0	0.0	0.0	0.0
0.1562	1.0	0.0	0.0	0.0

Antimicrobial with interpretative criteria using CLSI standard. Unit of zone of inhibition (mm) susceptible ≤ 4µg/mil or 20mm.

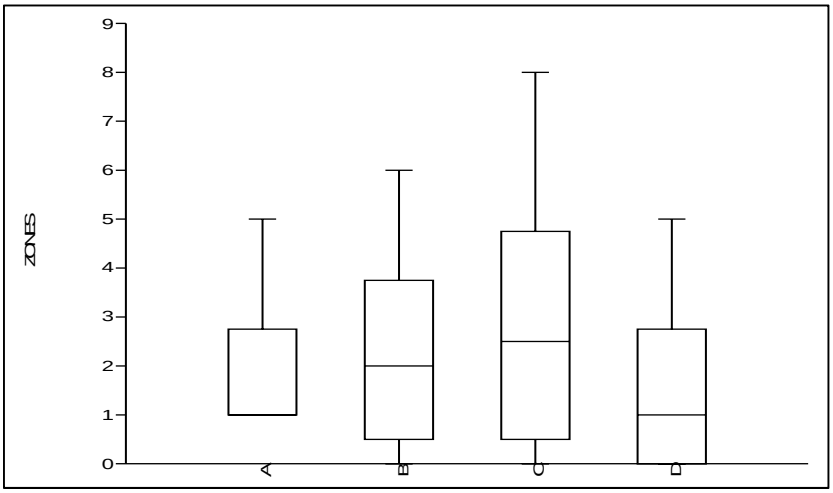


Fig 6: Graphical representation of Fraction two antibacterial activities of ethyl acetate leaf extract using ethyl acetate to elute.

Table 3.7: Fraction three antibacterial activities of ethyl acetate leaf extract using Ethanol to elute.

Concentrations (mg/ml)	*Staphylococcus aureus*	*Candida albicans*	*Klebsiella pneumonia*	*Escherichia coli*
20	7.0	6.0	5.0	9.0
10	5.0	4.0	3.0	7.0
5	3.0	3.0	2.0	5.0
2.5	2.0	2.0	2.0	3.0
1.25	2.0	0.0	1.0	2.0
0.625	1.0	0.0	1.0	2.0
0.3125	1.0	0.0	1.0	1.0
0.1562	0.0	0.0	0.0	0.0

Unit of zone of inhibition (mm) Antimicrobial with interpretative criteria using (Clinical Laboratory Science Institute).Susceptible ≤ 4 Susceptible breakpoint is 4µg/mil or 20mm Intermediate 8─16, Resistant breakpoint is 32µg/mil or 14m, Resistant >32

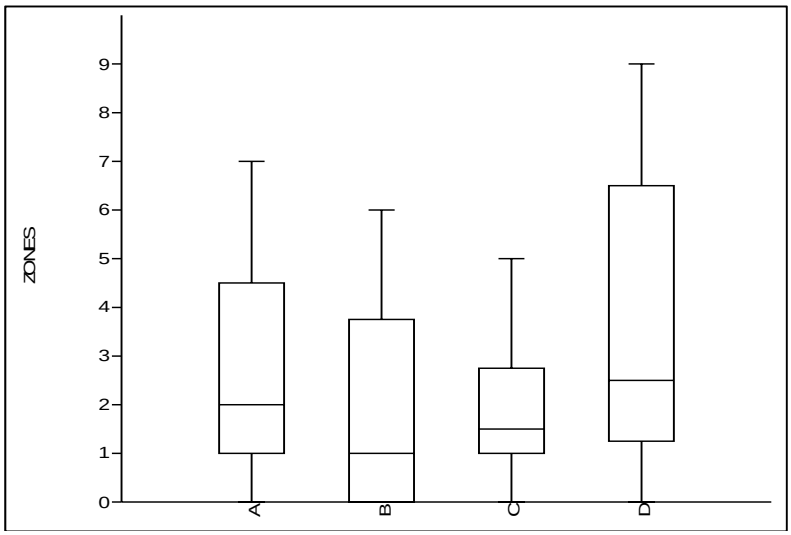


Fig 7: Graphical representation of Fraction three antibacterial activities of ethyl acetate leaf extract using Ethanol to elute.

Table 3.8: Qualitative Analysis of the Photochemical Screening of Medicinal plants

SAMPLE	Alkaloid	Cardiac Glycoside	Steroid	Anthraquinone	Phenol	Tannins	Saponin	Flavonoids
Erythrina senegalensis (Bark)	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	- ve
Erythrina senegalensis (Leaf)	+ ve	+ ve	+ ve	ND	+ ve	+ ve	+ ve	+ ve

Table 3.9: Quantitative Analyses of Minerals Present in Plant Extract (mg/100g)

Plant sample used	Na	K	Ca	Mg	Zn	Fe	Pb	Cu	Mn
Erythrina senegalensis (Bark)	20.00	26.19	31.49	22.08	25.07	5.75	ND	0.01	6.22
Erythrina senegalensis (Leaf)	21.35	26.00	32.98	21.37	24.98	7.86	N.D	0.02	7.76

Table 3.10: Quantitative Analyses of Anti –nutrients present in Plant Extracts Result in Percentage (%)

Parameters	BARK Erythrina senegalensis	LEAF Erythrina senegalensis
Tannin	2.64	2.62
Phenol	3.48	3.44
Phylate	1.25	1.20
Oxalate	1.50	1.00
Saponin	10.26	11.23
Flavonoids	7.92	7.90
Alkaloids	5.32	ND

Table 3.11: Quantitative analyses of Proximate Nutrient Composition of *Erythrina senegalensis* Extracts

Plants	% Ash	% MC	% CP	% Fat	% Fibre	%CHO
Erythrina senegalensis (F1)	8.75	7.35	16.19	5.42	8.55	53.74
Erythrina senegalensis (F2)	7.21	6.34	14.83	5.43	9.42	50.12

Discussion

The antimicrobial activities of ethyl acetate bark extract using N hexane to elute during the chromatographic process shows that *Candida albicans* is susceptible while *Klebsiella pneumonia* having the least value of zone of inhibition is not susceptible. *Staphylococcus aureus* and *Escherichia coli* are less susceptible. According to Soro *et al*., 2010, it was stated that *Erythrina senegalensis* extracts is active against *Candida albicans* which is a causative agent of Candidiasis.

Antimicrobial activities of ethyl acetate bark extract using ethyl acetate to elute shows that Staphylococcus aureus is susceptible followed by *Klebsiella pneumonia* while *Candida albicans* is less susceptible. This result is in accordance to Wagate *et al*., 2010, which state that Staphylococcus aureus has the highest sensitivity which is an indication that Gram-positive bacteria are more susceptible to antimicrobial effects of *Erythrina senegalensis* than Gram-negative bacteria. These results can be explained by the fact that Gram-positive bacteria are devoid of outer membrane in their cell walls. Thus the outer membrane may be responsible of the difference observed in the sensitivity level between Gram-positive and Gram-negative bacteria. The antimicrobial activities of ethyl acetate bark extract using ethanol to elute; it shows that *Escherichia coli* and *Klebsiella pneumonia* are more susceptible than *Candida albicans*.

Antimicrobial activities of ethyl acetate leaf extract of the plant using N hexane to elute reveals that *Escherichia coli* is more susceptible than *Staphylococcus aureus* while *Klebsiella pneumonia* is resistant. The resistance of Gram-negative bacteria towards antimicrobial agents is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharides molecules, presenting a barrier to penetration of numerous antibiotics molecules. (Shan *et al.*, 2007).

The antimicrobial activities of ethyl acetate leaf extract using ethyl acetate to elute shows that *Klebsiella pneumonia* is more susceptible than *Candida albicans*. *Staphylococcus aureus* and *Escherichia coli* are less susceptible.

Escherichia coli have the highest susceptibility followed by *Klebsiella pneumonia*. *Staphylococcus aureus* is more susceptible than *Candida albicans* in the antimicrobial activities of ethyl acetate using ethanol to elute.

The MIC values were generally lower than the MBC values against the test organisms showing that the extracts are bactericidal in action.

It can therefore be deduced from the results of the tables that bark extract of *Erythrina senegalensis* extracted with ethyl acetate using ethyl acetate to elute, and the of leaf extract of *Erythrina senegalensis* extracted with ethyl acetate using ethanol to elute shows an increase in the effectiveness of the antimicrobial activities than that of N hexane. This is due to the fact that different solvents have different polarities hence different degrees in solubility for the various phyto-constituents (Marjorie, 1999), thus accounting for this disparity in activity between the solvents used. The study also showed that the activity of the extracts is concentration dependent. Several factors ranging from concentration of antimicrobial agent, initial population density of the organisms, their growth rate and the rate of diffusion into the medium affects the activity of antimicrobials (Hugo and Russel, 1998; Prescott *et al.,* 2002). The extracts were active against both the bacteria and fungi studied. The broad spectrum of activity exhibited shows that *E. senegalensis* has potential for novel drug source that will serve as chemotherapy for various infections.

Phytochemical screening of the extracts in this study revealed that *E. senegalensis* contained some active chemical compounds (Saponins, tannins, glycosides, phenols and alkaloids). The presence of secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs (Mann *et al.,* 2008) and therefore explains its traditional use as health remedy. Tannins and Saponins may be responsible for the haemostatic activity of plant as they have been reported to arrest bleeding from damage vessels by precipitating proteins to form vascular plugs (Okoli *et al*., 2007). Flavonoids are said to be antioxidants and free radical scavengers which prevent oxidative cell damage and has equally been reported to have strong anticancer activity as well as possess the ability to inhibit tumor growth. It equally lowers the risk of developing heart diseases (Okwu and Okwu, 2004).

The mineral composition of the bark and leaf of *Erythrina senegalensis* show that sodium(Na), Potassium(K), Calcium(Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), Copper (Cu) and Manganese (Mn) were all present except lead which was not determined. Calcium has the highest amount of mineral composition in both bark and leaf of the plant with (31.49) and (32.98) respectively. Copper has the least mineral composition in both bark and leaf with (0.01) and (0.02) respectively.

The anti-nutrient analysis of *Erythrina senegalensis* extracts shows that Saponins has the highest value in both Bark leaf with 10.25% and 11.23% respectively, followed by Flavonoids which has 7.92% and 7.90% respectively. Phylate and oxalate have the lowest value in both leaf and bark while alkaloids are not determined in the leaf extract. With my result and the previous study by (Sato *et al.,* 2004) [37], it was revealed that Erybraedin A, a Flavonoids isolated from many *Erythrina* species is an antimicrobial agent and has been shown to have a strong activity against yeast pores and also showed a high growth inhibitory potency against vanomycin resistant enterococci (VRE) and multiresistant *Staphylococcus aureus* (MRSA), these antibacterial activities were based on bacteriostatic action. Senegalensein, a flavanone isolated from the stem bark of *E. senegelensis* has been reported to exhibit a HIV-inhibitory activity and an antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis* (Dastidar *et al*., 2004). Flavonoids have been reported to exert multiple biological effects including antimicrobial, cytotoxicity and anti-inflammatory (Yenjai *et al.,* 2004; Furuta *et al.,* 2004) [47, 8].

Analysis of the proximate nutrient composition of *Erythrina senegalensis* extract shows the presence of ash, moisture content, crude protein, fat, fiber and carbohydrate in the leaf and bark extract of *Erythrina senegalensis*. Carbohydrate has the highest composition while fat has the lowest composition in both leaf and bark.

Conclusion

Man has benefited from the exploitation of medicinal plants over years for curing of diseases even before the discovery of antibiotics.

It can be concluded from the research that *Erythrina senegelensis* play an important role in the treatment of different diseases and this is due to the fact that the chemical composition is active against microorganisms that cause diseases.

Recommendation

It is thereby recommended that man should explore and purify more of medicinal plants such as the one studied; *Erythrina senegalensis* to fight against public health problems. Also, more solvents should used to extract the plant in order to determine other compositions and concentration of elemental components of the plant, and that more tested organisms should be used in the antimicrobial activities.

Onsent: It is not applicable.

Ethical Approval: It is not applicable.

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Reference

1. Ahmedulla M, Nayar MP. Red data book of Indian plants: Calcutta: Botanical Survey of. 1999, 582.
2. Al-Harrasi A, Al-Rawahi A, Hussain J, Rehman N, Ali L, Hussain H. Proximate analysis of the resins and leaves of Boswellia sacra. J Med Plants Res. 2012; 6(16):3098-3104. *Antimicrob Agents* 12(3):257-262
3. Bako SS, Madu PC. Ind. J Bot Res. 2007; 3(1):17-22.
4. Dastida SG, manna A Kumar, Mazumdar KA, Dutta K, Chakrabarty NK, Motohashi AN, Shirataki NY. Studies on the antibacterial potential of isoflavones inter. J antimicro Agent. 2004; 23:99-102.
5. Donfack JN, Njayou FN, Rodrigue TK, Chuisseu DDP, Tchana NA, Finzi VP *et al.* Evaluation of antimicrobial potentials of stem bark extracts of *Erythrina senegelensis* DC. Afr. J microbiol Res. 2010; 4(17):1836-1841.

6. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constiuents of some Nigerian medicinal plants. Afr J Biotechnol. 2005; 4(7):685-68.
7. Ekpo MA, Etim PC. Antimicrobial activity of ethanolic and aqueous extracts of *Sida acuta* on microorganisms from skin infections. J Med Plant Res 2009; 3(9):621-624. Encyclopedia Britannica, 2010 Anthelmintic. Encyclopedia Britannica Ultimate Reference Suite, Chicago.
8. Furuta T, Kimura T, Kondo S, Wakimoto T, Nukaya H, Tsuji K, Tanaka K. Concise total synthesis of flavone C-glycoside having potent anti-inflammatory activity. Tetrahedron. 2004; 60:9375-9379.
9. Griggs, Govendarajan, Turmeric-Chemistry, Technology and Quality. CRC Food Sci. Nutr. 2001; 12:199-301.
10. Guevarra BQ. A guidebook to plant screening phytochemical and biological. UST Publishing House, Philippines. Hawkins EB., Ehrich SD, 2006 Goto Kola. University of Maryland Medical Center, Baltimore, USA, 2005.
11. Hill, Hidaka K, Masuda T, Yamaguchi H. Chemical studies on antioxidant mechanism of curcumin analysis of oxidative coupling products from curcumin and linoleate. J Agri Food Chem. 1989; 49:2539-2547.
12. Horwitz W. Editor. Official methods of analysis of AOAC international. 17th ed. USA: Association of Official Analytical Communities, 2003.
13. Hugo WB, Russell AD. Pharmaceutical Microbiology. (6th Edition). Oxford, 1998; 91-100.
14. Hussain J, Rehman N, Al-Harrasi A, Ali L, Ullah R, Mabood F *et al*. Nutritional prospects and mineral compositions of selected vegetables from Dhoda Sharif-Kohat. J Med Plants Res. 2011; 5(29):6509–6514.
15. Hussain HS, Deeni YY. Plants in kano ethomedicine; screening for antimicrobial activity and alkaloids. International Journal of Pharmacognosy, 1991; 29(1):51-56, ISSN 0976-8858.
16. Idu M, Omogbai EkI, Aghimien GEI, Amaechina F, Timothy O, Omonigho SE. Preliminary, Phytochemistry, aantimicrobial properties and acute toxicity of *Stachytarpheta jamaicensis* (L). Vahl. Leaves. Trends in Medicinal Research. Inc. New York, 811. 2007; 2:193-198.
17. Iwu MW, Duncan DR, Okunji CO. New antimicrobials of plant origin. In, 1999.
18. Kone WM, Solange KE, Dosso M. Assessing Sub-saharian Erythrina for efficacy: traditional uses, biological activities and phytochemistry. Pak J Biological Sci. 2011; 14(10):560-571.
19. Lee J, WK Oh, Ahn JS, Kim YH, Mbafor JT, Wandji J *et al*. Prenyliso flavonoids from *Erythrina senegalensis* as novel HIV-1 protease inhibitors. *Planta Med.* 2009; 75:268-27.
20. Mahato SB, Sen S. Advances in triterpenoid research: 1990-1994. Phytochemistry. 1997; 44:1185-1236.
21. Maheshwari JK, Singh KK, Saha S. Ethnobotany of tribals of Mirzapur District, Uttar Pradesh, Economic Botany Information Service, NBRI, Lucknow. 1986.
22. Mann A, Banso A, Chifford LC. Antifungal properties of Angogeissusleiocarpus and Terminaliaavicennioides. Tanzania Journal of Health Research. 2008; 10:34-38.
23. Mann A, Yahaya Y, Banso A, John F. Phytochemical and antimicrobial activity of *Terminalia avicennioides* extracts against some bacteria pathogens associated with patients suffering from complicated respiratory tract diseases. J Med Plants Res. 2008; 2(5): 94-97.
24. Marjorie MC. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 1999; 12(4):564.
25. Mary J. Lobelia herb treats respiratory problems and more. Available at: http://ezinearticles.com/?Lobelia-Herb-Treats-Respiratory-Problems-and-More&id=1. 2008; 484636.
26. Ngadjui TB, Moundipa FP. Study of hepatoprotective and antioxidant fraction from *Erythrina senegalensis* stem bark extract in vivo pharmacology online 1:120-130.
27. Oakenfull D, Sidhu G. 1990. Saponins, A useful treatment for hyperchlesterolemia. Europ J Clin Nutr. 2008; 44:79-88.
28. Okoli CO, Akah PA, Okoli AS. Potentials of leaves of *Aspilia africana* (Compositae) in wound care: An experimental evaluation. BMC Comp. Alt. Med. 2007; 7:24-30.
29. Okwu DE, Okwu ME. Chemical composition of *Spondias mombin* Linn plant parts. J Sustain Agric Environ. 2004; 6(2):140-147.
30. Okwu DE. Phytochemicals, Vitamins and Mineral contents of two Nigeria Medicinal plants. Int. J Mol Med Adv Sci. 2005; 1(4):375-381.
31. Osuntokun Oludare Temitope. Evaluation of antimicrobial activities of crude ethyl acetate and ethanol extracts of *Morinda lucida* leaf, stem, and bark growing in Adekunle Ajasin University Botanical Garden against selected clinical isolates, World Journal of Biomedicine and Pharmaceutical Sciences, Biosciences Research Support Foundation https://www.brsfoundation.org/wjbps. 2015; 1(1):6-14.
32. Osuntokun Oludare Temitope, Olajubu FA, Olorunnipa TA, Thonda OA. Elemental Composition, Evaluation of Anti-nutrients, and Antioxidant Potentials of Morinda lucida, European Journal of Medicinal Plants. 2016B; 12(4):1-10, 2016, Article no. EJMP.23081 ISSN: 2231-0894, NLM ID:101583475, DOI:10.97 34/EJMP/2016/23081
33. Osuntokun Oludare Temitope, Oluduro AO, Odubanjo OV, Dada TO. Evaluation of Antibacterial Potency of Crude Ethyl Acetate and Ethanol Extracts of Morinda lucida Leaf, Stem and Bark on Mycobacterium Species, Isolated from the Chest Hospital, Obafemi Awolowo Teaching University, Ile-Ife, Osun State, Nigeria, Journal of Advances in Medical and Pharmaceutical Sciences. 2016A; 5(3):1-8. 2016, Article no. JAM PS. 20705 ISSN: 2394-1111 SCIENCEDOMAIN international l, www.sciencedomain.org.
34. Prescott LM, Harley JP, Klein DA. Microbiology. 5th edition. McGraw-Hill Companies, 2002.
35. Rahe, Van Stoden, Benny Antory. Isolation purification and identification of curc uminoids from turmeric (*Curcuma longa*) by column chromatography. J Exp Sci. 2000; 2:21-25.
36. Salie Simon A. Inhibitory effect of curcuminoids on MCF 7 cell proliferation and structure-activity relationship. 1996; 129:111-116.
37. Sato M, Tanaka H, Oh-uchi T, Fukai T, Etoh H, Yamaguchi R. Antibacterial activity of phytochemicals isolated from *E. zeyheri* against vanomycin-resistant enterococci and their combinations with vanomycin. Phytotherapy Res. 2004; 18(11):906-910.
38. Sofowara Schieffer GW. Pressurized liquid extraction of

curcuminoids and curcuminoid degradation product from turmeric (*Curcuma longa*) with subsequent HPLC assay. J liq Chromatogr Related Technol. 2002; 25:3033 3044.
39. Sofowora A. Medicinal Plants and Traditional medicine in Africa. 1st ed., New York: John Wiley and Sons Ltd. 1982; 168-171.
40. Togola A, Austarheim I, Theis A, Diallo D, Paulsen BS. Ethno-pharmacological uses of *Erythrina senegalensis*: a comparison of three areas in Mali, and link between traditional knowledge and modern biological science. J Ethnobiol. Ethnomed. 2008; 4:6. DOI: 10.1186/1746-4269-4-6
41. Trease GE, Evans MC. Textbook of pharmacognosy, 12th ed. Tindall, London 1983; 343-38.
42. Ugbogu OC, Onyeagba RA, Chigbu OA. Lauric acid content and inhibitory effect of palm kernel oil on two bacterial isolates and *Candida albicans*. African Journal of biotechnology. 2000; 5(11):1045-1047.
43. Van Wyk BE, Gericke N. People`s plants. A guide to useful plants of Southern Africa. Briza Publicaation Pretoria, 2000.
44. Wagate CG, Mbaria JM, Gakuya DW, Nanyingi MO. Kareru PG. 2010. Screening.
45. Wandji J, Fomum ZT, Tillequin F, Baudouin G, Koch M. Expoxyiso flavones from *Eryhtrinasenegalensis*. Phyto chem. 1994; 35:1573-1577.
46. Won Keun Oh, Hyun Sun Lee, Soon Cheol Ahn, Jong Seog Ahn, J. Tanyi Mbafor, Jean Wandji *et al.* Prenylatediso flavonoids from *Erythrina senegalensis*. Phyto chem. 1999; 51:1147-115.
47. Yenjai C, Prasanphen K, Daodee S, Kittakoop P. Bioactive flavonoids from *Kaempheriliaparvin flora*. Fitoterapia. 2004; 75:89-92.
48. Sofowora A. Medicinal plants and traditional medicine in Africa published in association with spectrum Books Ltd. Ibadan by John Wiley and Sons, NY, 1984, 142-143.
49. Musa AM, Aliyu AB, Yaro AH, Magaji MG, Hassan HS, Abdullahi MI. Preliminary phytochemical, analgesic and anti-inflammatory studies of the methanol extracts of *Anisopus mannii* (N.E. Br) (Asclepiadaceae) in rodents. African Journal of Pharmacy and Pharmacology 2009; 3(8):374-378.
50. Doughari JH, Human IS, Bennade S, Ndakidemi PA. Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. Journal of Medicinal Plants Research 2009; 3(11):839-848.
51. De N, James NE. Antibacterial spectrum of extracts of *Ocimum gratissimum* L (Basil) ND *Xylopia aetiopica* A. Rich (Dunal). Nigerian Journal of Applied Science. 2002; 11:165-175.
52. Sani D, Sanni S, Ngulde SI. Phytochemical and antimicrobial screening of the stem aqueous extracts of Anisopus mannii. Journal Medical Plant Resource. 2009; 3(3):112-115.