Assessment of Antibacterial, Phytochemical Properties and GCMS Profiling of Crude *Polyalthia Longifolia Extract*

Oludaretemitope Osuntokun[1], Olanbiwonnu A. A[2], Orimolade G. F[3]

[1&3]Department of Microbiology, Faculty of Science, AdekunleAjasin University, AkungbaAkoko, P.M.B 001, Ondo State, Nigeria

[2]Department of Microbiology, Faculty of Natural & Applied Science, Ajayicrowther University, Oyo,, Oyo State, Nigeria.

Abstract— *The purpose of this present study is to determine the antibacterial potency of essential oil of Polyalthialongifolia, to determine the presence of phytochemicals and structural determination ofbioactive component from the leaf portion using GCMS. In this present study, the antibacterial activity from the essential oils of Polyalthialongifolia leaf and stem bark against seven bacteria (gram-positive and gram-negative) which are Staphylococcus aureus, Bacillussubtilis, Salmonellatyphi, Klebsiellapneumoniae, Escherichia coli, Proteus vulgaris and Pseudomonas aeruginosa known to be pathogenic to human causing high mortality among human. This study involved the extraction of essential oils from air-dried leaf and stem bark of Polyalthialongifolia using soxhlet method with n- hexane as the extracting solvent. Antibacterial activity was carried out using agar well diffusion method. Both plants part essential oil showed high antibacterial property. The sensitivity test revealed that Staphylococcus aureushas the highest zone of inhibition (18.0mm) to Polyalthialongifolia stem bark essential oil at 100mg/ml while the least zone of inhibition (3.0mm)was recorded for Escherichia coli at 100mg/ml to the essential oil of Polyalthialongifolialeaf.However, phytochemical analysis of the plants revealed the presence of bioactive components such as cardiac glycoside, steroid, anthraquinone, flavonoid, phenol, alkaloid and reducing sugars. The presence of these components enhances the effectiveness of plants essential oil in treating various diseases and helps to act as an effective antimicrobial agent. The essential oil of Polyalthialongifolialeaf was further analyzed by gas chromatography–mass spectroscopy (GC–MS), the main constituents were 6-octadecenoic acid (30.75%), Diisooctyl phthalate (3.87%) 1,1,6-trimethyl-3-methylene (17%). The various phytochemical and GC-MS components found in this plant were responsible by and large for the antibacterial activitiesexhibited the essential oil of Polyalthialongifolia leaf and stem bark in this study.*

Keywords—Polyalthialongifolia,Essential oils, Antibacterial and Phytochemical activity.

I. INTRODUCTION

Polyalthialongifoliais an evergreen plantcommonly used as an ornamental street tree due to its effectiveness in combating noise pollution. It belongs to the kingdom: plantae, division: *Magnoliophyta*, Class:*Magnoliopsida*, Subclass: *Magnoliidae*, Order:*Mognoliids*, Family: *Annonaceae*, Tribe:*Annoneae*, Genus:*Polyalthia*, Species: *Longifolia*. *Polyalthialongifolia*is also known as falseAshoka, Buddha Tree, Green champa, Indianmast tree, and Indian Fire tree. It exhibitsymmetrical pyramidal growth with willowy weeping pendulous branches and long narrowlanceolate leaves with undulate margins. Thetree is known to grow over 30 ft in height. Intraditional medicines various herbal preparationsare being used for treating duodenal ulcers (Pradhan*etal*., 2011).

Evergreen tree can grow up to a height of 15-20 meters tall. Young plants have straight trunks and weeping pendulous branch. The longest branch is seen at the base and shorter at the end of the trunk, giving an appearance of conical crown. Leaves are long, narrow dark green and glossy. Leaf blades are ovate-oblong to ovate-lanceolate with wavy margins. Reticulate veins rose on both surfaces of leaf. Transverse section of the leaf through the midrib showed bowl shaped abaxial parts and straight adaxial side. Both the adaxial and abaxial epidermal layers were single layered thin walled cubical cells. The epidermal cells wide, polygonal, thin walled and the walls were straight or slightly wavy. The epidermal cells followed by four to six layers of angular collenchyma cells on both the sides. In the midrib region, vascular bundle is encircled by a schlerenchymatous ring. Bundle sheath, xylem and phloem are clearly visible

Inflorescences axillary, fasciculate and shortly pedunculate, racemose, or umbelliform and sessile, mostly many flowered. Flowers are delicate pale green with wavy petals. The flowers last for a short period, usually two to three weeks and are not conspicuous due to their color. Sepals are ovate-triangular, outside it is tomentulose but inside glabrous. Petals are greenish yellow, narrowly triangular-lanceolate. Stamens are; connectives apically convex. Carpels are 20-25 in number with one ovule per carpel; stigmas are sessile. Fruits are borne in clusters of 10-20, usually ovoid in shape. Initially fruits are green in color but turns purple or black when ripe. Seeds are pale brown, ovoid, with a longitudinal groove.The stem bark; roots and leaves have been studied for various biological activities, such as antibacterial, antidiabetic, anti-inflammatory, and antioxidant activity. In addition, the bioactive compounds of this plant and its pharmacological activities due to its geographic density are relatively new area for investigation (Ugochi*et al.*, 2011)

In traditional medicines various herbal preparations are being used for treating duodenal ulcers.*Polyalthialongifolia*is a tall handsome evergreen tree and it is cultivated all over India. The plant has been used in traditional system of medicine for the treatment of fever, skin diseases, diabetes, hypertension and helminthiasis.A number ofbiologically active compounds have been isolated from this plant. The plant extract and isolated compounds were studied for various biological activities like antibacterial activity, cytotoxicity, antifungal activity.

Stem bark, flower, leaf, root and fruit can be used as potential herbal samples in pharmacy as decoction.

Fig.1: Leaf of Polyalthialongifolia

Fig.1.1: Stem bark of Polyalthialongifolia

II. MATERIALS AND METHODS

Sample Collection

Mature leaf and Stem bark of *Polyalthialongifolia*were collected from the School environment (ETF750),AdekunleAjasin University AkungbaAkoko,Ondostate Nigeria,latitude (7.21692 North) and longitutide (5.21561 East) during the harmattan season, October, 2016 and taken to the school plant science and biotechnology lab for proper naming. The leaves were initially rinsed with distilled water and dried on paper towels in the room at room temperature for one week.

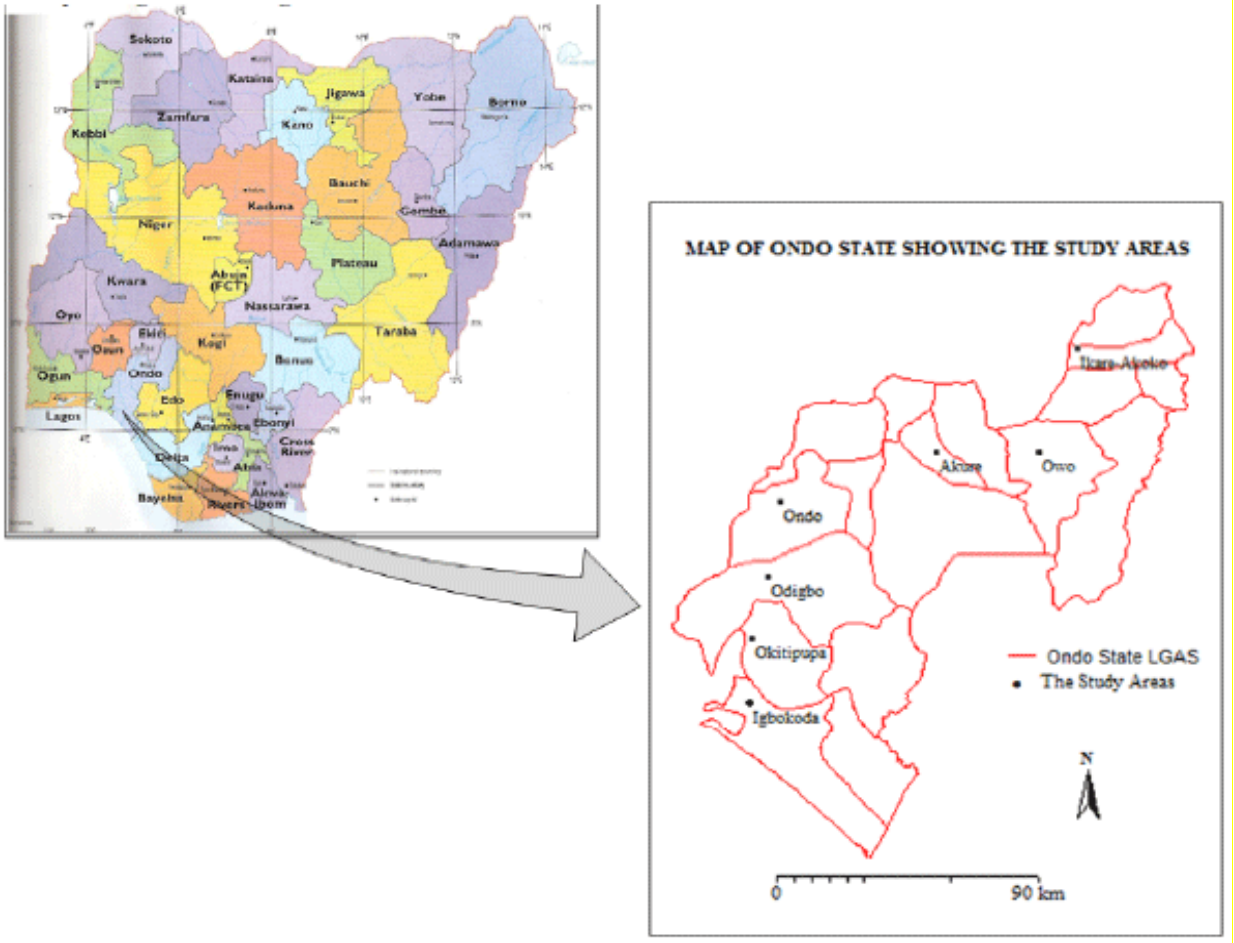


Fig.2.1: Map of Nigeria indicating AdekunleAjasin University AkungbaAkoko,Ondo state Nigeria,

Test Microorganisms

The test organisms used were standard strains of pathogenic bacteria. They include strains of *Staphylococcusaureus* (ATCC 55620), *Salmonella typhi* (ATCC) *Escherichia coli* (ATCC 23922),*Klebsiellapneumonia* (ATCC 15380),*Bacillus subtilis(*ATCC 11778*)Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 13325). The bacterial isolates were cultured in slanted Mueller Hinton agar in bijou bottles and transported at a low temperature to microbiology laboratory AdekunleAjasin University, AkungbaAkoko (AAUA), Ondo state and incubated in an incubator for reactivation of the bacteria. They were then sub-cultured and stored in the refrigerator for further tests (Osuntokun, 2015).

2.4.1 Preparation of test organisms

Slants of the various organisms were reconstituted using aseptic condition, using a sterile wire loop, approximately one isolated colony of each pure culture was transferred into 5ml of sterile nutrient broth and incubated for 24 hours. After incubation, 0.1ml of the isolated colony was transferred into 9.9ml of sterile distilled water contained in each test tube using a sterile needle and syringe, and then mixed properly. The liquid now serve as a source of inoculum containing approximately 10^6cfu/ml of bacterial suspension (Osuntokun, 2015).

Extraction Method of *Polyalthialongifolia*

About 250 ml of N-Hexane was poured into a round bottom flask. 10 g of the powdered bark and leaf sample was placed in the thimble and was inserted in the centre of the soxhlet extractor. The extractor was then heated to and held constant at 65°C. As the solvent begins boiling; the vapor rose through the vertical tube of the extractor into the condenser at the top of extractor. The liquid condensate then dripped into the filter paper thimble in the centre which contained the solid sample from which oil is extracted. The extract seeped through the pores of the thimble and filled the siphon tube, where it flowed back down into the round bottom flask. This was allowed to continue for 5 hours. It was then removed from the tube. Afterwards, the solvent was separated from the oil using rotary evaporator. Then

cooled in the desiccators and weighed again to determine the amount of oil extracted. Further extraction was carried out at 30 min intervals until the sample weight at further extraction and the previous weight became equal. At the end of the extraction, the resulting mixture containing the*Polyalthialongifolia*oil was heated to recover solvent from the oil (Musa *et al*., 2016).

2.6Antibacterial Sensitivity Test of *Polyalthialongifolia*

2.6.1 Preparation and Standardization of Inoculum Suspension

Direct colony suspension technique was used for the standardization and preparation of inoculum. Pure cultures of the test organisms were transferred into sterile screw-capped McCartney bottles containing normal saline (0.90% w/w) using a flamed inoculating loop. A suspension with a turbidity equivalent to 0.5 McFarland standards was also prepared at the same time to serve as a reference for turbidity. To achieve equal turbidity, both the reference and inoculum suspensions were placed against a white card with black stripes. Turbidity was observed with the unaided eye. Standardized inoculums were refrigerated(Osuntokun, 2017).

2.6.2 Preparation of antimicrobial (plant extract) concentrations

Pure N-hexane extracts of both the leaf and bark of *Polyalthialongifolia* were weighed to ascertain total yield after plant extracts. 1000mg (1g) of both plant extracts were dissolved in 10ml of solvent (containing 7.5ml of water and 2.5 ml of DMSO) to achieve a stock concentration of 100mg/ml.

$$Concentration\ (\frac{mg}{ml}) = \frac{weightofextract\ (mg)}{volumeofsolvent\ (ml)}$$

Six other sterile McCartney bottles (3 for each plant) containing 5ml of water were used to carry out two-fold dilutions (log_2). To achieve this, 5ml of the stock concentration was dispensed into a sterile McCartney bottle containing 5ml of water. This yielded a concentration of 50mg/ml. this procedure was repeated for each plant until a concentration of 12.5mg/ml was achieved for each plant extract. At the end, concentrations of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml of both leaf and bark of *Polyalthialongifolia*were obtained. These bottles were stored in the refrigerator at -4^{o}C (El Astal*et al.,* 2005).A standard antibiotic control was also prepared using the broad spectrum antibiotics ciprofloxacin (100µg).

2.6.3 Antimicrobial assay of *Polyalthialongifolia*

The agar diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA). The MHA plates were prepared by pouring 5 ml of molten media into sterile Petri-plates. The plates were allowed to solidify along with a 2.0 McFarland standard suspension of the test organism for 5 minutes. The different concentrations of extracts (12.5, 25, 50 and 100 mg/disc) were loaded onto the agar after boring 6mm holes using a cork borer. Theplates were kept for incubation at 37^0C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in duplicates (Osuntokun, 2014).

2.7 Phytochemical Analysis of *Polyalthialongifolia*

2.7.1 Qualitative Phytochemical Analysis

2.7.1.1 Test for Reducing Sugars

1ml of the plant extract was mixed with Fehling A and Fehling B separately; a brown color with Fehling B and a green color with Fehling A indicated the presence of reducing sugars.

Test for Alkaloids

Plant extracts were half diluted with NH_4OH and lixiviated with ethyl acetate for 24hours at room temperature. Theorganic phase was separated from the acidified filtrate and was basified with ammonium hydroxide (NH_4OH) at pH 11-12. It was then extracted with chloroform (3X), condensed by evaporation and submitted for chromatographic analysis. Alkaloidspots were separated into the elution phase using the solvent mixture chloroform and methanol (15:1). Appearance of orange spots after spraying with Dragendorff's reagents indicates a positive result(Mallikharjuna*et al.,* 2007).

Test for Anthraquinone (Borntrager's test)

About 50mg of plant extract was heated with 1ml of 10% ferric chloride ($FeCl_3$) solution and 1ml of concentrated hydrochloric acid, after which the extract was cooled and filtered. The filtrate was shaken with an equal amount of diethyl ether. Further extraction with concentrated ammonia was done to remove ethyl ether. The presence of a pink or deep red coloration of aqueous layer indicates a positive result (Kumar *et al.,* 2007).

Test for Cardiac glycosides (TLC method)

Powdered test samples were extracted with 70% ethanol using a rotary shaker (180 thaws/min) for 10hr. 70% lead acetate was added to the filtrate and centrifuged at 5000rpm for 10 min. Further centrifugation was done for the supernatant by adding 6.3% Na_2CO_3 at 10000 rpm for 10min. The retained supernatant was re-dissolved in chloroform and use for chromatography. Glycosides were eluted out using a solvent system that comprise Ethyl

acetate, Methanol, and water (in the ratio 80:10:10) (Onwukaeme*et al*., 2007).

Test for Flavonoid(TLC method)

1g of powdered plant extract was extracted with 10ml methanol on water bath (60°C for 5min). The filtrate was condensed and filtered by evaporation. A mixture of water and Ethyl acetate (10:1 ml) was mixed thoroughly. Retainedethyl acetate phase and use for chromatography. Elution was done by using chloroform and methanol (19:1) solvent mixture to detect flavonoid spot.The colour and hRf values of these spots were recorded under ultraviolet (UV254nm) light(Kumar et al., 2007).

Test for Saponin(TLC method)

Two grams of powdered test samples was added to 10 ml 70% Ethanol by refluxing for 10 min. The filtrate was condensed and enriched with saturated n-Butanol, and mix thoroughly. With the retained butanol, extract was condensed and used for chromatography. Saponinswere separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The colour (yellow) and hRf values of these spots were recorded by exposing eluted chromatogram to the iodine vapours (Mallikharjuna*et al.*, 2007).

Test for Steroid(TLC method)

Two grams of powdered test samples was dissolved in 10ml methanol in water bath (80°C for 15 min). The condensed filtrate was used for chromatography. The sterols were eluted using a solvent system of chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The colour and hRf values of these spots were recorded under visible light after spraying the plates with anisaldehyde-sulphuric acid reagent and heating (100°C for 6 min)(Mallikharjuna*et al.*, 2007).

Test for Tannin (Braemer's test)

To 10% alcoholic ferric chloride, 2-3ml of methanolic extract (1:1) was added. The appearance of a dark blue or greenish grey coloration of the solution indicates a positive result (Parekh and Chanda, 2007)

Quantitative phytochemical analysis of *Polyalthialongifolia*

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 mixtures were heated using a hot water bath. At about 55oC, for 4 hour with continuous stirring, after which the mixture were filtered and the residue re-extracted with 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 layers were recovered while the ether layer was discarded. The purification process was repeated three times. 60 rnl of n-butanol was added. The combined n-butanol extracts were washed twice with 10 m1 of 5% aqueous sodium chloride. The remaining solution was 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. (Obadoni*et al.,* 2001)

Flavonoids

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution were filtered through Whatman filter paper No 1. The filtrates were later transferred into a crucible and evaporated into dryness over a water bath; the dry content were weighed to a constant weigh. (Krishnaiah*et al*., 2009)

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 absorbances were measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract. (Harbourne, 2005).

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.(Harbourne, 2005).

GC-MS Analysis of Polyalthialongifolia

Polyalthialongifolia oil was analyzed using GC/MS (Shimadzu capillary GC-quadrupole MS system QP 5000) with two fused silica capillary column DB-5 (30 μm, 0.25 mm i.d, film thickness 0.25 μm) and a flame ionization

detector (FID) which was operated in EI mode at 70 eV. Injector and detector temperatures were set at 220°C and 250°C, respectively. One micro-liter essential oil solution in hexane was injected and analyzed with the column held initially at 60°C for 2 min and then increased by 3°C/min up to 300°C. Helium was employed as carrier gas (1 ml/min). The relative amount of individual components of the total oil is expressed as percentage peak area relative to total peak area. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds, or by retention indices (RI) and mass spectra (He, 2002).

III. RESULTS

Table 3.1 Shows the diameter of zone of inhibition of the extract oil of the stem bark of *Polyalthialongifolia*. The highest zone of inhibition was recorded on the plate containing *Staphylococcus aureus* at 100mg/ml concentration with 18mm zone of inhibition and 10mm zone of inhibition at 12.5mg/ml, followed by *Bacillus subtilis*with 14mm zone of inhibition at a concentration of 100mg/ml and 8mm zone of inhibition at 12.5mg/ml. it was observed that *Escherichia coli* and *Proteus vulgaris* have intermediate susceptibility with 10mm and 8mm respectively at 100mg/ml and 3mm and 2mm zone of inhibition respectively at a concentration of 12.5mg/ml. The least susceptible organism was found to be *Pseudomonas aeruginosa* with 5mm zone of inhibition at 100mg/ml and 3mm zone of inhibition at 50mg/ml concentration. The table also showed the positive control used in the study, which was 50mg/ml Ofloxacin.

Table 3.2shows the diameter of zone of inhibition of the leaf extract of *Polyalthialongifolia* leaf against the test organisms used in the study. It was observed from this table that *Staphylococcus aureus*was the most susceptible organism with 14mm zone of inhibition at a concentration of 100mg/ml, and 7mm zone of inhibition at a concentration of 6.25mg/ml. this was followed by *Bacillus subtilis*with 12mm zone of inhibition at a concentration 100mg/ml and 5mm zone of inhibition at 6.25mg/ml. *Salmonella typhi* has intermediate value, with 7mm zone of inhibition at 100mg/ml and 3mm zone of inhibition at 25mg/ml. the least susceptible organism to the leaf extract was observed to be *Escherichia coli* with 3mm zone of inhibition at 100mmg/ml and 1mm zone of inhibition at 6.25mg/ml.

Table.1: Antimicrobial assay of the stem bark of Polyalthialongifolia extract on selected clinical organisms

Concentrations (mg/ml)

ORGANISM	100	50	25	12.5	CTR (50mg/ml)
Staphylococcus aureus	18.0	15.0	12.0	10.0	28.0
Pseudomonas aeruginosa	5.0	3.0	0.00	0.00	0.00
Proteus vulgaris	10.0	7.0	5.0	0.00	28.0
Klebsiellapneumonia	8.0	6.0	4.0	2.0	19.0
Escherichia coli	10.0	7.0	5.0	3.0	24.0
Bacillussubtilis	14.0	12.0	10.0	8.0	22.0
Salmonella typhi	9.0	6.0	3.0	0.00	27.0

NOTE: CTR= Positive Control (50mg/ml Ofloxacin) **Diameter of zone of inhibition -mm**

Table.2: Antimicrobial assay of the leaf of Polyalthialongifolia extract on selected clinical organisms

ORGANISM	**100**	**50**	**25m**	**12.5**	**CTR(50mg/ml)**
Salmonella typhi	7.0	5.0	3.0	0.0	27.0
Pseudomonas aeruginosa	5.0	4.0	2.0	0.00	0.00
Staphylococcus aureus	14.0	12.0	10.0	0.00	28.0
Klebsiella pneumonia	5.0	3.0	1.0	0.00	19.0
Escherichia coli	3.0	1.0	0.00	0.00	24.0
Proteus vulgaris	5.0	2.0	0.00	0.00	0.00
Bacillussubtilis	12.0	10.0	8.0	5.0	22.0

NOTE: CTR= Positive Control,Control (50mg/ml Ofloxacin)

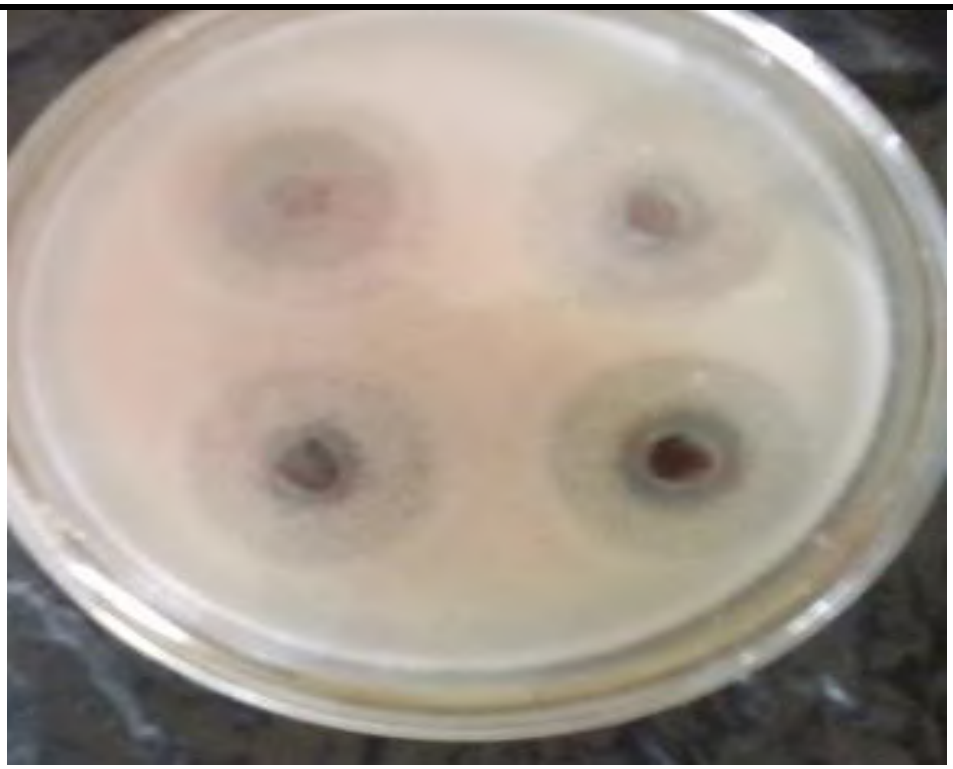


Plate.1: Diameter of zone of inhibition of the leaf extract of Polyalthialongifolia

Plate.2: Antibacterial activity of Polyalthialongifolia leaf against Staphylococcus aureus

T**able 3.3** shows the qualitative analysis of *Polyalthialongifolia*stem bark and leaf using methanol as solvent. The table revealed that alkaloids, tannins, saponin, and reducing sugar is positive in both bark and leaf of *Polyalthialongifolia* while cardiac glycoside is negative in both bark and leaf, steroids, phenol, and flavonoids is

present in bark but absent in leaf while anthraquinone is present in leaf and not detected in bark.

Table 3.4 Shows the qualitative phytochemical analysis of *Polyalthialongifolia* bark and leaf using ethyl acetate as solvent. It was revealed that cardiac glycoside, anthraquinone, phenol, tannins, and saponins are present in both bark and leaf of *Polyalthialongifolia*while alkaloids, steroids, and reducing sugar are present in bark but absent in leaf while flavonoids is absent in both barkand leaf.

Table 3.5 shows the qualitative phytochemical analysis of *Polyalthialongifolia* bark and leaf using dichloromethane as solvent. This revealed that cardiac glycoside, steroids, tannins, and saponins, is present in both bark and leaf of *Polyalthialongifolia* while alkaloids, phenol, and reducing sugar is present in bark but absent in leaf while anthraquinone and flavonoids is absent in both bark and leaf *of Polyalthialongifolia*

Table 3.6 shows the qualitative phytochemical analysis of *Polyalthialongifolia* bark and leaf using n-hexane as solvent presented in revealed that cardiac glycoside, anthraquinone, phenol, tannins and saponins are present in both bark and leaf of *Polyalthialongifolia*while alkaloids, steroids, flavonoids and reducing sugar are absent in bark but present in leaf of *Polyalthialongifolia.*

Table.3.3: Qualitative phytochemical analysis of Polyalthialongifolia (Methanol)

Sample	Alkaloid	Cardiac Glycoside	Steroids	Anthraquinone	Phenol	Tannins	Saponin	Flavonoids	Reducing sugar
Bark	+ ve	-ve	+ ve	ND	+ ve	+ ve	+ ve	+ ve	+ve
Leaf	+ ve	- ve	- ve	+ ve	- ve	+ ve	+ ve	- ve	+ve

NOTE: +ve = positive, -ve = Negative, ND = Not Detected

Table.3.4: Qualitative phytochemical analysis of Polyalthialongifolia(Ethyl acetate)

Sample	Alkaloid	Cardiac Glycoside	Steroids	Anthraquinone	Phenol	Tannins	Saponin	Flavonoids	Reducing sugar
Bark	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	- ve	+ ve
Leaf	- ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	- ve	- ve

NOTE: +ve = positive, -ve = Negative

Table.3.5: Qualitative phytochemical analysis of Polyalthialongifolia (Dichloromethane)

Sample	Alkaloid	Cardiac Glycoside	Steroids	Anthraquinone	Phenol	Tannins	Saponin	Flavonoids	Reducing sugar
Bark	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	-ve	+ ve
Leaf	- ve	+ ve	+ ve	- ve	- ve	+ ve	+ ve	-ve	- ve

NOTE: +ve = positive, -ve = Negative

Table.3.6: Qualitative phytochemical analysis of Polyalthialongifolia (N- hexane)

Sample	Alkaloid	Cardiac Glycoside	Steroids	Anthraquinone	Phenol	Tannins	Saponin	Flavonoids	Reducing sugar
Bark	- ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	- ve	- ve
Leaf	-+ve	+ ve	+ve	+ ve	+ ve	+ ve	+ ve	+ ve	-+ve

NOTE: +ve = positive, -ve = Negative

Table 3.7 Shows the quantitative phytochemical analysis of *Polyalthialongifolia* leaf and stem bark in percentage using methanol as the solvent. It was observed from the table that phytate has the highest value for both the leaf and stem bark with 6.49% and tannins was found to have and high value of 5.32 in the stem bark. Alkaloid was found to be the least compound in the stem bark with a value of 1.23%. it was observed that tannins has the second highest value in the leaf extract with 2.64%. the least phytoconstituent in the leaf was discovered to be oxalate, with a value of 2.10%, while saponin was not detected in the stem bark.

Table 3.8 Shows the quantitative phytochemical analysis of *Polyalthialongifolia* leaf and stem bark in percentage using ethyl acetate as the solvent. It was observed from the table that Alkaloid, oxalate, saponin and flovonoid all have the highest value the stem bark with 9.53% respectively and phytate was found to have the least value in the stem bark with 8.53%. Alkaloid and saponin were found to be the highest compounds in the leaf with value of 1.29% respectively. While phytate was observed to have the least value in the leaf, with a value of 1.23%.

Table 3.9 Shows the quantitative phytochemical analysis of *Polyalthialongifolia* leaf and stem bark in percentage using ethyl acetate as the solvent. It was observed from the table that Alkaloid, oxalate, saponin and flavonoid all have the highest value the stem bark with 9.53% respectively and phytate was found to have the least value in the stem bark with 8.53%. Alkaloid and saponin were found to be the highest compounds in the leaf with value of 1.29% respectively. While phytate was observed to have the least value in the leaf, with a value of 1.23%.

Table.3.7: Quantitative phytochemical analysis of Polyalthialongifolia(Methanol) %

Sample	Alkaloid	Oxalate	Phytate	Phenol	Tannins	Saponin	Flavonoids
Bark	1.23	1.25	6.49	4.31	5.32	ND	1.87
Leaf	2.20	2.10	6.49	2.37	2.64	2.62	2.34

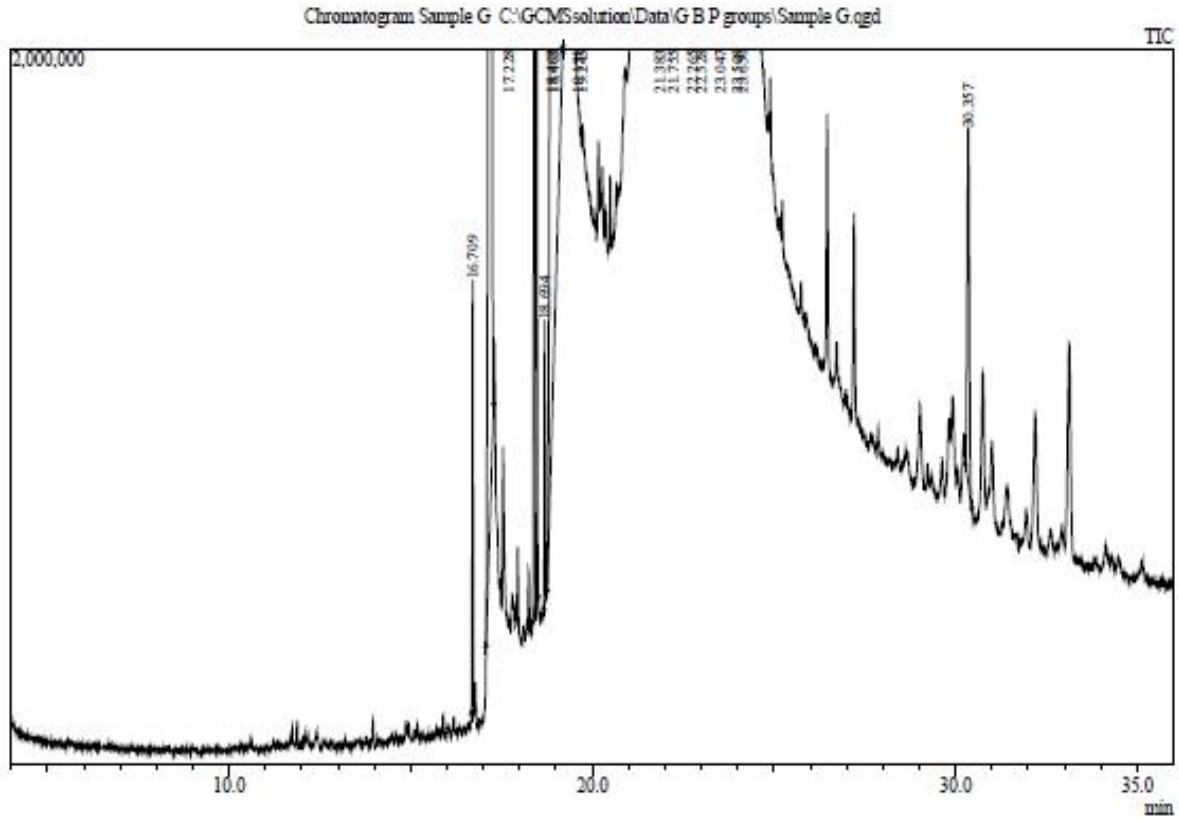
NOTE: ND = Not Detected

Table.3.8: Quantitative phytochemical analysis of Polyalthialongifolia(Ethyl acetate) %

Sample	Alkaloid	Oxalate	Phytate	Phenol	Tannins	Saponin	Flavonoids
Bark	9.53	9.53	8.53	8.55	9.23	9.53	9.53
Leaf	1.29	1.28	1.23	2.25	1,26	1.29	1.28

Table.3.9: Quantitative phytochemical analysis of Polyalthialongifolia(N- hexane) %

Sample	Alkaloid	Oxalate	Phytate	Phenol	Tannins	Saponin	Flavonoids
Bark	9.53	9.53	8.53	8.55	9.23	9.53	9.53
Leaf	1.29	1.28	1.23	2.25	1,26	1.29	1.28



Spectra.3.1: Mass Spectra of GCMS Analysis of Polyalthialongifolia

Table 3.10 shows the result of Fifteen volatile phytoconstituents which were found to be the most abundant in the n-hexane extract of the stem bark of *Polyalthialongifolia*. These constituents and their calculated percentage peak area compositions include hexadecanoic acid(1.18%) with a low retention time of 16.709, followed by pentadecanoic acid (7.455%) with retention time of 17.228, 9,12-octadecadienoic acid(z,z)(3.09%) at retention time of 18.402, 9-octadecenoic acid (z)-(4.26%) at retention time of 18.465, Methyl stearate(0.76%) at retention time of 18.694, 6-octadecenoic acid(30.75%) at retention time of 19.131, Octadecanoic acid(9.64%) at retention time of 19.245, 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetra(17.00%) at retention time of 21.383, azulene(1.30%) at retention time of 21.755, Diisooctyl phthalate(3.87%) at retention time of 22.265,But-3-enal(9.57%) at retention time of 22.528, 6.beta.Bicylo[4.3.0]nonane(1.46%) at retention time of 23.047, (7a-Isopropenyl-4,5-dimethyloctahydroinden(4.52%) at retention time of 23.508, Guaia-1(10), 11-diene(4.19%) at retention time of 23.650, and lastly the target compound 7,22-Ergostadienone(0.97%) at retention time of 30.275 was isolated after several chromatographic separation techniques, identified through GC-MS analysis.

Table.3.10a: Result showing the chemical constituent present in oil of Polyalthialongifolia using GC-MS analysis

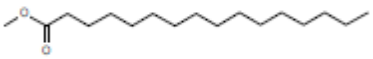
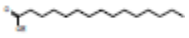
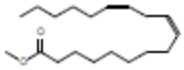
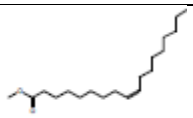
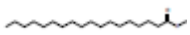
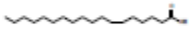
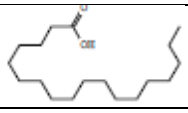
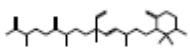
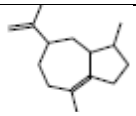
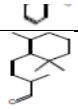
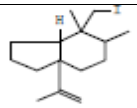
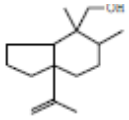
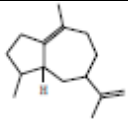
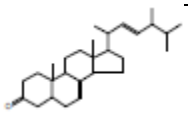
COMPOUND NAME	RETENTION TIME	MOLECULAR WEIGHT	BASE PEAK	HEIGHT(%)	STRUCTURE
Hexadecanoic acid	16.709	270	74.05	1.18	
Pentadecanoic acid	17.228	242	73.05	7.45	
9,12-octadecadienoic acid(z,z)	18.402	294	67.05	3.09	
9-octadecenoic acid (z)-	18.465	296	55.05	4.26	
Methyl stearate	18.694	298	74.05	0.76	
6-octadecenoic acid	19.131	282	55.05	30.75	
Octadecanoic acid	19.245	284	73.05	9.64	
1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetra	21.383	452	95.10	17.00	
Azulene	21.755	204	107.10	1.30	

Table 3.10b: Result showing the chemical constituent present in oil of *Polyalthialongifolia* using GC-MS analysis

D					
But-3-enal	22.528	206	123.15	9.57	
6.beta.Bicylo[4.3.0]nonane	23.047	332	95.10	1.46	
(7a-Isopropenyl-4,5-dimethyloctahydroinden	23.508	222	111.05	4.52	
Guaia-1(10), 11-diene	23.650	204	107.10	4.19	
7,22-Ergostadienone	30.275	396	269.15	0.97	

NOTE: See Appendix for other information on the identified compounds and the library.

IV. DISCUSSION

Herbal medicine in developing countries is commonly used for the traditional treatment of health problems. In recent years multiple drug resistance in human pathogenic microorganisms has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases, making it a global growing problem (Davis, 1994).Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infections obtained from various sources such as medicinal plants.(Ahmmad, 1998).

Essential Oils are slightly more active against gram-positive than gram-negative bacteria (Delaquis*et al.,* 2002; Pintore*et al.,* 2002; Harpaz*et al.,* 2003). It was observed from this study that the essential oil of *Polyalthialongifolia* stem bark showed antibacterial activity against all the test bacteria used in the study. *Staphylococcus aureus* was observed to be the most susceptible organism to the essential oil extract of the stem bark having 18mm zone of inhibition at 100mg/ml and 10mm zone of inhibition at 12.5mg/ml. this was followed by *Bacillus subtilis* with 14mm zone of inhibition at 100mg/ml and 8mm zone of inhibition at 12.5mg/l. *Pseudomonas aeruginosa*was observed to have the least susceptibility to the essential oil extract of this plant with 5mm zone of inhibition at 100mg/ml and 3mm zone of inhibition at 50mg/ml. this goes in line with the finding of Parekh, 2007,who reported susceptibility of *staphylococcus aureus* to the essential oil extract of *Polyalthialongifolia.*

It was observed from the essential oil extract of the leaf of Polyalthialongifolia that Staphylococcus aureus was the most susceptible organism with 14mm zone of inhibition at 100mg/ml and 7mm zone of inhibition at 12.5mg/ml. the least susceptible organism to the essential oil extract of the leaf was Escherichia coli with 3mm zone of inhibition at 100mg/ml and 1mm zone of inhibition at 50mg/ml. this goes in line with the observation of Harpaz et al., 2003 who also reported the antimicrobial activity of the essential oil of Polyalthialongifolia against some pathogens.

The antimicrobial activity of this *Polyalthialongifolia.*could be attributed to the presence of certain bioactive components such as alkaloid, tannins and flavonoid. It could also be due to the fact that the solvent (N-Hexane) used in the study was able to extract the active bio constituents present in *Polyalthialongifolia.*,and which is able to exert antimicrobial effect against the tested bacteria. Also, the antibacterial property could be because of the concentration used in the study, i.e. 100mg/ml concentration was enough to exert antibacterial effect against the test bacteria

The phytochemicals tested are known to exhibit medicinal activity and physiological activity. Alkaloids are an important drug source and have been reported to possess antimicrobial, antioxidant, and cytotoxic activity (Rahman*et al.,* 2009). Medicinally, tannins are used in antidiarrhoeal, haemostatic, and antihaemorrhoidal preparation. Saponins are glycosides of triterpenes, steroids or steroidal alkaloids found in plants. They are useful in lowering cholesterol (hypocholesterolemic property), as antioxidants, and as antidiabetic and anti-inflammatory agent.

Generally, majority of the secondary metabolites studies and flavonoid in leaf and stem bark parts of Polyalthialongifolia have present with higher amount in methanolic extract than that of the other solvents. However, flavonoids and saponins were rich in ethyl acetate extracts. It is explained that the polarity level and species nature are playing major role in extracting the secondary secondary metabolites (Ghasemzadeh et al., 2011).

The qualitative phytochemical screening of *Polyalthialongifolia*extracts of bark and leaf using methanol (Table 3.3) revealed that the crude extracts contains alkaloids, phenol, tannis, saponins and reducing sugar. Anthraquinone is not detected in the bark but present in the leaf extract, while flavonoids is present in the bark but absent in the leaf, steroids is present in the bark of the plant but absent in leaf. In Table 3.4, the qualitative phytochemical analysis of *Polyalthialongifolia* leaf and stem bark using ethyl acetate, from this table, it was observed that cardiac glycosides, anthraquinones, phenol, tannins, saponins are present in both bark and leaf extract. Alkaloids is present in bark but absent in leaf extract, steroids is present in bark but absent in leaf. Also reducing sugar is present in bark but absent in leaf. Dichloromethane was also used as solvent and this revealed that cardiac glycoside, steroids, tannins, saponins are present in boththe bark and the leaf extract while alkaloids is present in bark but absent in leaf and reducing sugar is present in bark and absent in leaf this goes in line with the observation of Singh *et al.,* 2002.

These secondary metabolites are reported to have many biological and therapeutic uses, so this plant is expected to have many medicinal uses. Phytochemical screening in using n-hexane as the solvent revealed the presence of cardiac glycosides, anthraquinone, phenol, tannins, saponins. While alkaloids is absent in bark but present in leaf, flavonoids is present in leaf but absent in bark.

Reducing sugar is absent in bark but present in leaf (Harbourne, 2005).

Quantitative phytochemical screening shows that phytate is higher in methanolic extract of bark and leaf of Polyalthialongifolia while the lowest is alkaloid of bark extract and saponin is not detected in the stem bark of the plant. It was observed that methanol extract registered the higher percentage of yield. It may be due to high polarity of methanolic solvent which can draw high variety of plant constituents than the other solvents did (Pulsamy*et al*., 2011).

Steroids display analgesic properties (Rupasinghe*et al*., 2003; Sayyah*et al*., 2004, Malairajan*et al.*, 2006; Tamil Selvan*et al., 2012*). Glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia and are found as secondary metabolites in medicinal plants Someanthraquinones have been reported to possess antiosteoporotic activity (Li *et al.,* 2009).

This study revealed that the extract of *Polyalthialongifolia* contained many important phytochemical constituents with various medicinal properties. The presence of biologically important phytochemicals in the *Polyalthialongifolia* extracts may contribute to their reported medicinal values and indicates that it is a potential source of the development of drugs (Edeoga*et al.,* 2005).The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes.

Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant; such as phenols (Kazmi*et al*.,1994), essential oils (Cosentino*et al.*,1999). terpenoids (Habtemariam*et al*.,1993; Taylor *et al*.,1996), alkaloids (Omulokoli*et al*.,1997) and flavonoids (Batista *et al*.,1994). Preliminary phytochemical analysis during the present study also ascertains the presence of some potential group of bioactive substances (Dafererra*et al*.,2003).

Compounds present in oil of Polyalthialongifolia include long chain polyunsaturated fatty acids (hexadecanoic acid) are attracting attention as potential new topical treatments for gram-positive infections due to their antimicrobial potency and anti-inflammatory properties. The compounds are bactericidal. Fatty acids are attracting attention as potential therapeutic antimicrobial agents due to their potency, broad spectrum of activity and the lack of classical resistance mechanisms against the actions of these compounds (Desbois, 2010).In particular, various long-chain polyunsaturated fatty acids (LC-PUFAs), which are found naturally at high levels in many marine organisms (Berge *et al*., 2005).

Fifteen peaks were detected in the GC-MS Chromatograms of *Polyalthialongifolia* bark extract. There were two antimicrobial compounds that were present in considerable amount(47.75%) and another thirteen antimicrobial compounds comprised of (52.05%). A component hexadecanoic acid present in the oil has antimicrobial properties. Other minor compounds such as pentadecenoic acid, methyl stearate, diisoocty phthalate has anti oxidant activity. Azulene has antifungal activity.

6- octadecenoic acid which is the most abundant has antimicrobial activity possess antibacterial, antifungal and antioxidant activity, anticarcinogenic-exist in human blood and urine and sense as endogenous peroxisome proliferator as also reported by Kumar *et al*., 2010.Hexadecanoic acid as reported by Mahmood*et al*., 2009 revealed that it possesses antifungal, antioxidant, hypocholesterolemicnematicide, pesticide, haemolytic, 5-Alpha reductase inhibitor, potent antimicrobial activity.Diisoocty phthalate also as reported by Kumar et al., 2010 revealed thatit possesses antifungal, antibacterial, antiviral, and antioxidant activities.The medicinal activity of Polyalthialongifolia bark is attributed to the antimicrobial compounds present in the bark extract (Desbois, 2010).

V. CONCLUSION

In conclusion, *Polyalthialongifolia* possess phytochemicals which offers potential antibacterial property against the selected clinical isolates. The study ascertains the presence of some potential group of bioactive substance. *Polyalthialongifolia* is moreso a medicinally important herb used in the traditional system of medicine and an ancient remedy to be explored for novel therapeutic uses. *Polyalthialongifolia* also contains essential oil which is useful in treatment of some infections. Therefore it is concluded that *Polyalthialongifolia* oil should be further studied phytochemically to elucidate the active principle in the bark and leaf in which can be used as a leading antibacterial agent.

REFERENCES

[1] Pradhan, P., Jhajharia, M., Chulet, R., and Yagnik, D. (2011).Pharmacologyonline; 2: 11-15.

[2] Ugochi O., Joshua P.E., and Omeh, O.V. (2011).AntioxidantProperties of Polyalthialongifolia. New York Science Journal. 2011; 4(6), 83-87.

[3] Osuntokun, O.T. (2015). Comparative Study of Antibacterial and Phytochemical Properties of Nigerian Medicinal Plants on Salmonella bongori and Salmonella enteritidis isolated from Poultry Feaces in Owo Local Government. Ondo State, Nigeria. Archives of current research international. 2(1): 1-11

[4] Musa,U., Mohammed, A.G., Mohammed, U., Garba, U.S., and Alhassan, B. (2015). Extraction of Chrysophyllumalbidum Seed Oil: Optimization and Characterization.Chemical and Process Engineering Research Journal, Vol. 30, pp. 252-253

[5] Edeoga, H. O., Okwu, D. E., and Balbic, B. O. (2005). "Phytochemical constituents of some Nigerian medicinal plants," Afr. J. Biotechnol. 4(7), 685-688.

[6] El-Astal, Z.Y., Ashour, A.E., and Kerrit, A.A.M. (2005). Antimicrobial activity of some medicinal plant extracts in Palestine. PAK. J. Med. 21(2): 187-193.

[7] Mallikharjuna, P.B., Rajanna, L.N., Seetharam, Y.N., and Sharanabasappa, G.K. (2007). Phytochemical studies of StrychnospotatorumLinn. *A medicinal plant.E-J. Chem.* 4, 510 - 518.

[8] Onwukaeme, D.N., Ikuegbvweha, T.B., and Asonye, C.C. (2007).Evaluation of phytochemical constituents, antibacterial activities and effect of exudates of Pycanthusangolensis (Myristicaceae) on corneal ulcers in rabbits. Trop. J. Pharm. Res. 6, 725-730. 9) Kumar et al., 2007

[9] Parekh, J., and Chanda, S.V. (2007). In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk. J. Biol. 31: 53-58.

[10] Harborne, J. B. (2005).Phytochemical methods -- A guide to modern techniques of plant analysis Springer Pvt. Ltd.

[11] Li, N., Qin, L.P., Han, T., Wu, Y.B., and Zhang, H.(2009). "Inhibitory effects of Morindaofficinalis extract on bone loss in ovariectomized rats, " *Molecules* 14(6),2049-2061.

[12] Singh, G.,Kapoor, I.P.S.,Pandey, S.K., Singh, U.K.*et al.* (2002). "Studies on essential oils: Part 10; Antibacterial activity of volatile oils of some spices". Phytotherapy Research. 16 (7): 680–682.

[13] Ahmad, Z., and Mohammad, F. (1998). "Screening of Some Indian Medicinal Plants for their Antimicrobial Properties," *Journal of Ethnopharmacology*, Vol. 62, No. 382, pp. 183-193.

[14] Batista, O., Duarte, A., Nascimento, J., and Simones, M.F.(1994). Structure and antimicrobial activity of diterpenesfrom the roots of Plectranthushereroensis.*Journal of Natural Product* 57: 858–861.

[15] Cosentino, S., Tuberoso, C.I.G., Pisano, B., Satta, M.,Mascia, V., Arzedi, E., and Palmas, F. (1999). In vitroantimicrobial activity and chemical composition ofSardinian thymus essential oils. Letters in Applied Microbiology 29: 130–135.

[16] Davis, J. (1994). "Inactivation of Antibiotics and Dissemination of Resistance Genes," *Science*, Vol. 264, No. 5157,pp. 375-382.

[17] Delaquis, P.J., Stanich, K., Girard, B., and Mazza, G. (2002).Antimicrobialactivity of individual and mixed fractions of dill, cilantro,coriander and eucalyptus essential oils. International *Journal ofFood Microbiology* 74, 101–109.

[18] Desbois, A.P., and Smith, V.J.(2010). Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl. Microbiol. Biotechnol. 85, 1629–1642.

[19] Daferera, D.J., Ziogas, B.N., and Polissiou, M.G. 2003.Theeffectiveness of plant essential oils in the growth ofBotrytiscinerea, Fusarium sp. and Clavibactermichiganensis subsp. michiganensis. Crop Protection 22:39–44.

[20] Ghasemzadeh, A., Jaafar, H. and Rahmat, A. (2011). Effect of solvent types on phenolic an flavonoids content and antioxidant activities in two varieties of young ginger (Zingiberofficinale Roscoe.) extract. Journal of medicinal plant research. 5(7):1147-1154.

[21] Harpaz, S., Glatman, L., Drabkin, V., and Gelman, A. (2003). Effects of herbal essential oils used to extend the shelf life of fresh waterreared Asian sea bass fish (Latescalcarifer). Journal of Food Protection 66 (3), 410–417.

[22] Habtemariam, S., Gray, A.I., and Waterman, P.G. (1993).Anew antibacterial sesquiterpene from Premnaoligotricha.Jornal of Natural Product 56: 140–143.

[23] Kazmi, M.H., Malik, A., Hameed, S., Akhtar, N., and Ali,S.N. (1994). An anthraquinone derivative from Cassiaitalica.Phytochemistry 36: 761–763.

[24] Kumar, P.P., Kumaravel, S., and Lalitha. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of Vitexnegundo. Afr. J. Biochem.Res.4, 191-195.

[25] Mahmood, A., Ahmed, R., and Kosar, S.(2009). Phytochemical screening and biological activities of the oil components of Prunusdomestica Linn. J. Saudi Chem. Soc. 13, 273-277.

[26] Malairajan, P., Gopalakrishnan, G., Narasimhan, S., and Veni, J.K.(2006). "Analgestic activity of some Indian medicinal plants," J. Ethnopharmacol. 19(3), 425-428.

[27] Omulokoli, E., Khan, B., and Chhabra, S.C. (1997).Antiplasmodial activity of four Kenyan medicinal plants.Jornal of Ethnopharmacology 56: 133–137.

[28] Paulsamy, S. and Jeeshna M.V. (2011).Preliminary phytochemistry and antimicrobial studies of an endangered medicinal herb. Research journal of biological chemical science. 2(4):447- 457.

[29] Pintore, G., Usai, M., Bradesi, P., Juliano, C., Boatto, G., Tomi, F., Chessa, M., Cerri, R.,and Casanova, J. (2002). Chemical composition and antimicrobial activity of Rosmarinusofficinalis L. oils from Sardinia and Corsica.*Flavour and Fragrance Journal* 17, 15–19.

[30] Rahman. S., Akbor, M. M., Howlader, A., and Jabbar, A.(2009). "Antimicrobial and cytotoxic activity of the alkaloids of amalaki (Emblicaofficinalis)," Pak. J. Biol. Sci. 12(16), 1152- 1155.

[31] Rupasinghe, H.P., Jackson, C.J., Poysa, V., Di-Berado, C., Bowley, J. D., and Jenkinson, J. (2003). "Soyasapogenol A and B distribution in soybean (Glycine max L. Merr) in relation to seed physiology, genetic variability and growing location," J. Agric. Food chem..51(20), 5888- 5894.

[32] Taylor, R.S.L., Edel, F., Manandhar, N.P., and Towers,G.H.N. (1996). Antimicrobial activities of southern Nepalese medicinal plants. Journal of Ethnopharmacol50: 97–102.

[33] Selvan, R.T., Mohideen, A.K., Sheriff, M. A., and Azmathullah, N.M.(2012). "Phytochemical screening of Acalyphaindica L. leaf extracts, " Int. J. Appl. Technol. 3(2), 158-161

[34] Osuntokun OT, Olajubu FA. Comparative study of phytochemical and proximate analysis of seven Nigerian medicinal plants.Applied Science Research Journal.App. Sci. Res. J Pon Publishers.Ekpoma Edo state Nigeria. 2014;2(1):10-26.

[35] Obadoni BO, Ochuko, PO Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Glob. J. Pure Appl. Sci. 2001;86:2003-2008.