In Vitro Analysis, Secondary Metabolite Screening and GC-MS Profile of *Ricinodendron Heudelotii*(Muh.Arg) Essential Oil Extracts Against Selected Multiple Drug Resistance Clinical Isolates

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Abstract*:-Essential oils are valuable natural products used as raw materials in many fields and can be define as a volatile and liquid aroma compounds from natural sources usually plants. They may be found in different parts of plant, some could be leaves, seed, flower, peel, and Stem bark. This study aims at screening the antimicrobial property from the essential oil extracted from *Ricinodendron huedelotii* fresh seed and stem bark using soxhlet method with two distinct solvents(N-hexane and ethyl acetate). Antibacterial activity was carried out using well agar diffusion technique against multiple resistance clinical isolates. The N-hexane oil extracts from *Ricinodendron heudelotii* seed revealed *Bacillus subtilis and Pseudomonas aeruginosa* having the widest zone of inhibition (15.0mm) at 100mg/ml while the least zone of inhibition (1.0mm) was recorded for *Escherichia coli*at 12.5mg/ml with essential oil ethyl acetate extracts from *Ricinodendron huedelotii*stem bark. Phytochemical analysis of the plant showed the present of active components such as cardiac glycoside, steroid, flavonoid, phenol, alkaloid, reducing sugar,tannin, saponin, pyrrolizidine, terpenoid and volatile oil. The present of these components enhances the effectiveness of plant essential oilin treating various diseases and helped to act as an antibacterial agent. Essential oil extracted from *Ricinodendrn huedelotii* seed using two solvents was further analysed by gas chromatography – mass spectrophotometer. The main constituents were Oxycycloheptadec 8-en-one (20.48%), Pentadecanoic acid (11.95%), n-propyl 9,12-Octadecadienoic acid (6.40%), Octadesetrienoic acid ethyl ester (10.30%), Ascorbic acid(11.27%), Gamolenic acid (17.74%), Linolenic acid, methyl ester (6.74%) and Eicososatrienoic acid (8.94%).Those components aid the antibacterial activities of essential oil from the seed and stem bark of *Ricinodendron heudelotii.* The results obtained suggest that the *Ricinodendron heudelotii* essential oil can serve as an effective antibacterial agent.

Keywords: **In Vitro Analysis, Secondary Metabolite, *Ricinodendron Heudelotii,* Multiple Drug Resistance Clinical Isolates,**

I. INTRODUCTION

Pure essential oils are derived from various parts of the plants. These essential oils have a very high commercial value due to its properties. They are widely used in the various fields of industries, such as perfumery industries and pharmaceuticals. Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition (1). An essential oil defines as a more or less volatile material isolated from an odorous plant of a single botanical species by a physical process. They are oxygenated derivatives of hydrocarbon terpenes such as aldehydes, ketones, alcohols, phenols, acids, ethers and esters. Some terpenes are potent drugs against diseases such as heart disease, malaria and cancer (2). Essential oils are complex mixtures comprising many single compounds. Each of these constituents contributes to the beneficial or adverse effects of these oils. Therefore, the intimate knowledge of essential oil composition allows for a better and specially directed application. Considering all the aforementioned differences in essential oil composition, it is clear that only a detailed knowledge of the constituents of an essential oil will lead to a proper use in cosmetics by perfumers and cosmetic chemists. However, such a detailed knowledge can only be obtained by means of carefully performed capillary-GC experiment (3). *Ricinodendron heudelotii* is a fast-growing tree, reaching up to 50 m in height and 2.7 m in girth; bole straight with short buttress; bark grey, smooth at first becoming scaly with ageing; slash dark red, densely mottled with scattered pits and orange stone-cell granules. It has various local names, English (ground nut tree, cork wood, "African wood-oil nut tree, African wood, African nut tree); French (bois jasanga); German (afrikanisches Mahagoni); Swahili (muawa); Trade name (musodo, erimado, corkwood); Yoruba (erimado). The plant is known to have two subspecies, namely, *heudelotii* , and africanum (Mull. Arg.) . Subspecies *heudelotii* is known to be common in Senegal and Benin, while the subspecies africanum is predominant in the southern part of Nigeria and South Africa. *Ricinodendron heudelotii* is a species which belongs to the Euphorbiaceae family. (4). The fruit is an indehiscent yellow-greenish drupe, 2 to 3 lobed, generally spherical, 3-5 cm long and 2.5-4 cm width and weight ranges from 19 to 47 g. Fruits smell of overripe apples. The fruit has a fleshy mesocarp and a woody endocarp, and contain 2-3 seeds. Flowering takes place in April-May and ripening of fruits occurs in September and October. Fruits are produced

in large quantities. Dispersal is mainly gravitational but dispersal by bats, hornbills and rodents. Once the fruits have fallen on the ground, fruit pulp rots away. Afterwards, seeds remain dormant for a period of six months up to more than two years (5). *Ricinodendron heudelotii* has four major use categories including consumption, medicinal, sociocultural and soil fertility improvement. It is valued for its distinctively flavored seeds, commonly called "njansang" which are dried and used as flavoring and thickening agent in food. *Ricinodendron heudelotii* stem bark is used to treat yellow fever, anemia, malaria, stomach pain and disease of infants. It is also used as aphrodisiac in parts of Cameroon and in pregnancy to ease delivery (Fondoun et al 1-999). *Ricinodendron heudelotii* kernels are characterized by their high oil content (45- 55 %) and crude proteins (55.37 % in the defatted cake). The fatty acids composition of this oil indicates a high a- eleostearic acid content (52 % of total fatty acids) (6).

Fig 1.1: Showing *Ricinodendron heudelotii* tree (7)

Fig 1.2: Showing *Ricinodendron heudelotii* fresh fruits and rotten friuts (8).

Fig 1.3: Showing *Ricinodendron heudelotii* kernels with cracks (9,10)

Ricinodendron heudelotii (Njangsa), have positive benefits on certain physiological processes, and their inclusion in the diet may be desirable. These fatty acids have been shown to possess antitumor properties, improve cardiovascular functions, reduce adiposity and reverse oxidative stress due to heavy metal poisoning. The species of Njangsa is characteristic of secondary forest (10) and has a wide variety of uses. The wood is used for making household implements, drums and carvings and the bark is used to treat both elephantiasis and leprosy.

Seeds are used for multiple purposes. In Sierra Leone, they are used in rattles for bundu dances, while in Cameroon they are used in musical instruments as well as for local games: 'songo' in Cameroon and 'okwe' in Nigeria. The hard endocarp contains seeds which are the edible part of the fruits. Fruit pulp and endocarp are usually discarded when seeds, or more specifically seed kernels, are extracted. Most often kernels are dried and used as a condiment in recipes. Crushed njansang is used to thicken and favour soups, stews and other dishes. They are also cooked with chicken and vegetables or eaten plain. In addition, kernels may be roasted, made into a paste and used for making a sauce similar to peanut sauce (11).

II. MATERIALS AND METHODS

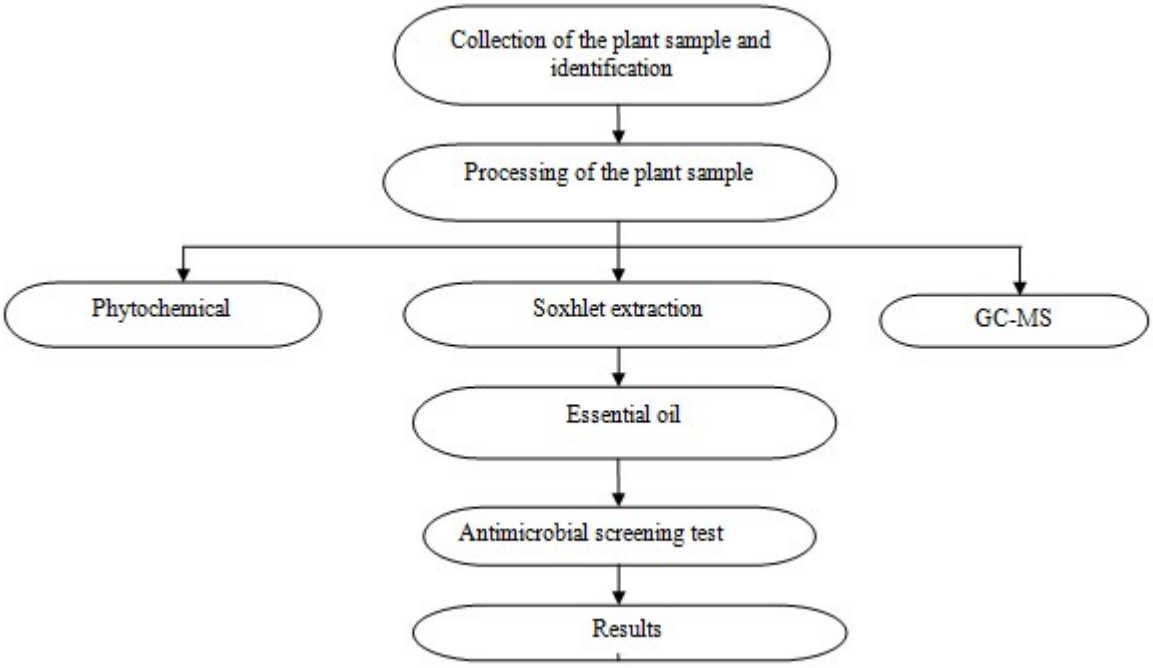


Chart 2.0 : Summary of methodology procedure.

2.2 Sample Collection

The seeds of *Ricinodendron heudelotii* (Mull. Arg.) were collected from an open area in Adekunle Ajasin University Akungba, Ondo State, Nigeria. The plant materials were rinsed in water, and then tied in a sack for about one week to enhance quicker decay of the seeds. The *Ricinodendron heudelotii* (Mull. Arg.) fruits are allow to fall from the trees, gather them into piles, and allow them to get rotten for 5-8 weeks. Once rotten, the seeds still within their shell are washed to prevent that the rotten organic matter will blacken kernels during further processing. Next, they are subjected to one or two consecutive boiling sessions that are intended to crack the seed shells. These cracks in the seed shell enable the kernels to be extracted using a sharp object such as a knife or nail. The kernels were then removed from the shells and grinded using an electric blender into coarse powder. The wet stem bark was peeled off from the tree and was cut into pieces to increase the surface area.

2.3 Identification of the sample

The plant was identified and authenticated at the department of Plant Science and Biotechnology, Faculty of Science, Adekunle Ajasin University Akungba.

2.4 Extraction of essential oil from Ricinodendron heudelotii.

Seeds can be processed to obtain oil. About 150g of the crushed kernels were measured and put into a thimble for exhaustive Soxhlet extraction using N-hexane and ethyl acetate as solvent. 150g of each samples was placed into the thimble of soxhlet apparatus. About 250ml of solvent was placed in the round bottom flask subjected to minimum heat using heat mantle for 3hours. The resultant mixture of solvent and essential oil was passed through a lie big condenser cooled by a continuous flow of fresh iced water. The oil then separated in the round bottom flask and turned into the samples bottles. The procedure was repeated until a sufficient amount of oil for analysis and antibacterial test was obtained. The oil is light yellow, with a pleasant taste resembling that of groundnut oil and it has been suggested that the high oil content of the seed together with the high proportion of poly-unsaturated fats in the oil, indicates its suitability for commercial production of cooking oil and margarine as well as for soaps and pharmaceutical preparations and the oil obtained was stored away from light prior to analysis. (12).

Fig 2.1: Soxhlet Apparatus (12).

2.5 Antibacterial Activity of Ricinodendron heudelotii (Mull. Arg.)

Anti-bacterial activity of the essential oil was tested using agar well diffusion method. The gram-negative bacteria used were *Escherichia coli, Klebsielle pneumonia, Pseudomonas aeruginosa, Protues mirabilis,* and *Salmonella typhii* while gram-positive bacteria were *Staphylococcus aureus* and *Bacillus* spp. The test organisms were inoculated in nutrient broth and incubated for 4-6 hours for 37^0C. To standardize the microbial inoculums for the susceptibility test, a barium chloride standard equivalent to McFarland No.0.5 standard. Antibacterial activity assay was done on Mueller Hinton agar. The media were reconstituted using solvent used for the extraction of the essential oil and sterilized by autoclaving at 121^0C for 15minutes then dispensed into petri dishes aseptically and left to solidify. The fresh grown microbial cultures were inoculated on solid media. A sterile cork-borer was used to bore well on the agar, test oil (1ml) of different concentration were dispensed into each well aseptically. All these procedures were done in dublicate. The inoculated plates were incubated at 37^0C for 24hours before the activity was determined. The activity of the test oils was established by the presence of measurable zones of inhibition (mm). The essential oil was tested for anti-bacterial activity(13).

2.6 Secondary metabolites (Phytochemical) analysis of Ricinodendron heudelotii (Mull. Arg.)

Qualitative Method of phytochemical Analyses

Plant filtrate were prepared by boiling 20 g of the fresh plant in distilled water. The solution was filtered through a vacuum pump. The filtrate were used for the phytochemical screening for flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides.

(i)Test for Alkaloids

About 0.2gram were 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 indicate the present of Alkaloids (14).

(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 (15).

(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 (15).

(iv) Test for Anthraquinones

One milliliter of the plant filtrate were 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 (16).

(v)Test for Anthocyanosides

One milliliter of the plant filtrate were mixed with 5 m1 of dilute HCI; a pale pink colour indicates the positive test (17).

(vi) Test for Flavonoids

One milliliter of plant filtrate were 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 (18).

(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 (19).

(viii) Test for Cyanogenic glucosides

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 (20).

(ix) Test for Cardiac glucosides

Legal test and the killer-kiliani was adopted, 0.5g of the extract were added to 2ml of acetic anhydrate plus H_2S0_4 (21).

Quantitative Method of Analyses of Ricinodendron heudelotii (Mull. Arg.)

(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 were heated using a hot water bath. At about $55^{O}C$, 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 separatory funnel and 20 rnl of diethyl ether were added and then shaken vigorously. The aqueous layer were 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 solution 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 (22).

(ii) 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 42. The filtrate were later transferred into a crucible and evaporated into dryness over a water bath; the dry content were weighed to a constant weight (23).

(iii) Cardiac glucosides

Legal test and the killer-kiliani wwas adopted, 0.5g of the extract were added to 2ml of acetic anhydrate plus H_2S0_4 (24).

(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 were measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract (25).

(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 (26).

(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 (27).

2.7 GC-MS Analysis of Ricinodendron heudelotii (Mull. Arg.)

A gas chromatography is a chemical analysis instrument for separating chemicals in a complex sample. A gas chromatograph uses a flow-through GC analysis narrow tube known as the column, through which different chemical constituents of a sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the stationary phase. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (retention time). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, column length and the temperature (28*)*. *Ricinodendron Heudelotii* oil was analysed using GC/MS (Shimadu capillary GC-quadrupole MS system QP 5000) with two fused silica capillary column DB-5 (30µm, 0.25mm i.d, film thickness 0.25µm) and a flame ionization detector (FID) which was operated in EI mode at 70 cV. Injector and detector temperatures were set at $220^{0}C$ and $250^{0}C$, respectively. One micro-liter essential oil solution in hexane was injected and analysed with the column held

initially at 60^0C for 2 mins and then increased bt $3^{0C/}$min up to 300^0C.Helium was employed a carrier gas (1ml/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 (29,30).

III. RESULT

Chart 3.1- Chart 3.4: Antibacterial activity of *Ricinodendron heudelotii* (Mull. Arg.) (seed) essential oil using N-hexane as solvent.

Chart 3.1 shows the zone of inhibition of test organisms by the essential oil extract of *Ricinodendron heudelotii* (seed) using N-hexane as solvent expressed in mm with *Bacillus subtilis* and *Pseudomonas aeruginosa* showing the widest zone of inhibition (15.0mm) while the least zone of inhibition (4.0mm) was recorded for *Klebsiella pneumonia*. The essential oil of *Ricinodendron huedelotii* (seed) act as a strong antibacterial agent against *Bacillus subtilis*, *Klebsiella pneumonia*, *Salmonella typhi, Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis* in-vitro. The oil showed activity against both gram positive and gram negative bacteria. On the average, antibacterial activity of essential oil was more pronounced on gram positive bacteria(mean zone of inhibition: 13.0mm) than gram negative (mean zone of inhibition 11.1mm).

Chart 3.5 – Chart 3.8: Antibacterial activity of *Ricinodendron heudelotii* (Mull. Arg.) (seed) essential oil using ethyl acetate as solvent.

Chart 3.2 Indicate that the essential oil of *Ricinodendron huedelotii* (seed) using ethyl acetate as solvent for the extraction of the oil were found to be active against only one gram positive *(Klebsiella pneumonia)* and two gram negative bacteria *(Salmonella typhi* and *Proteus mirabilis*) as shown by the inhibition zones in the table below. The *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella epidermidis* and *Escherichia coli* are resistance to the oil since no inhibition zones were observed.on the average, antibacterial activity of essential of *Ricinodendron huedelotii* (seed) using ethyl acetate as solvent was more pronounced on gram positive bacteria (zone of inhibition:11.0mm) than gram negative bacteria(mean zone of inhibition:10.0mm)

Chart 3.1 Measurement of zone of inhibition of *Ricinodendron heudelotii* essential oil (Seed) using N-hexane solvent against multidrug resistance isolates.

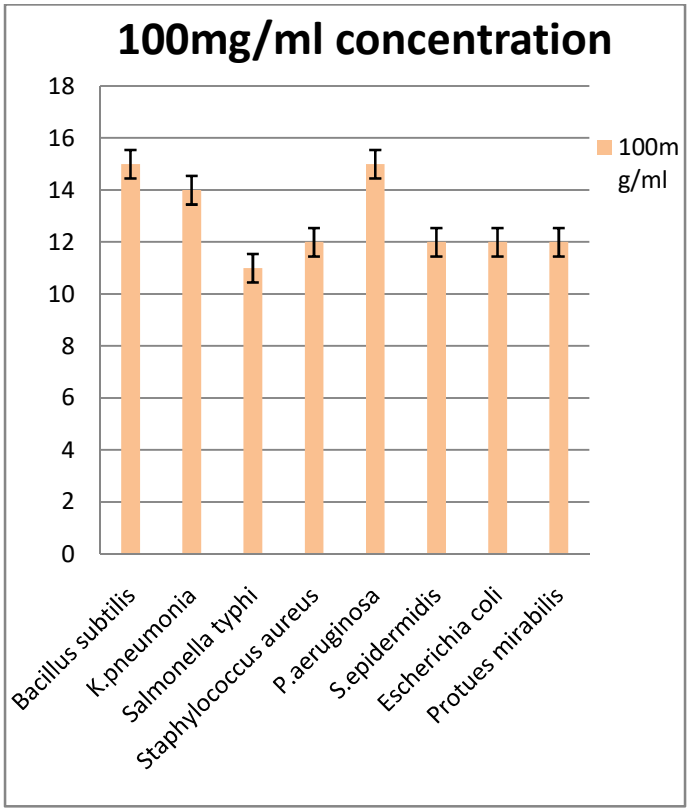


Chart 3.2.Inhibition zone of *Ricinodendron. heudelotii* essential oil (Seed) using N- hexane solvent against multidrug resistance isolates.

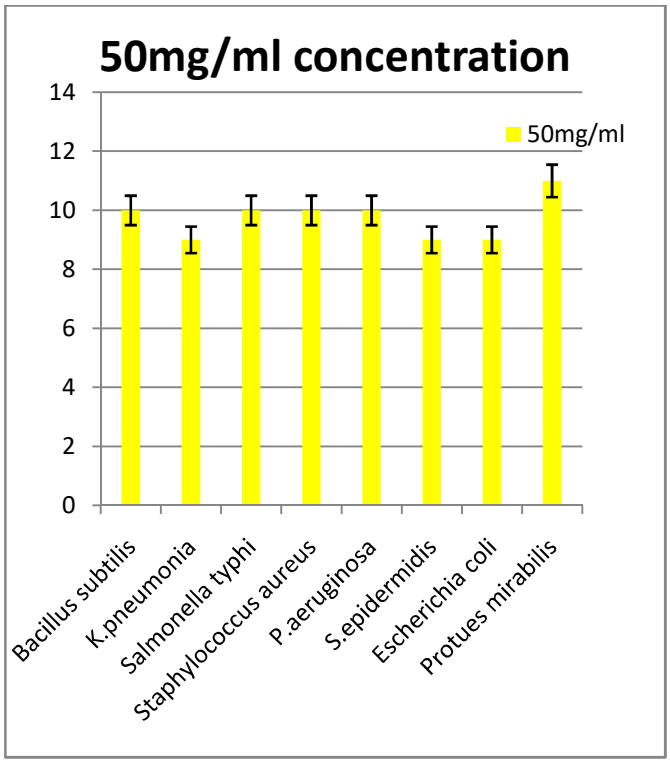


Chart 3.3 Inhibition zone of *Ricinodendron heudelotii* essential oil (Seed) using N- hexane solvent against multidrug resistance isolates.

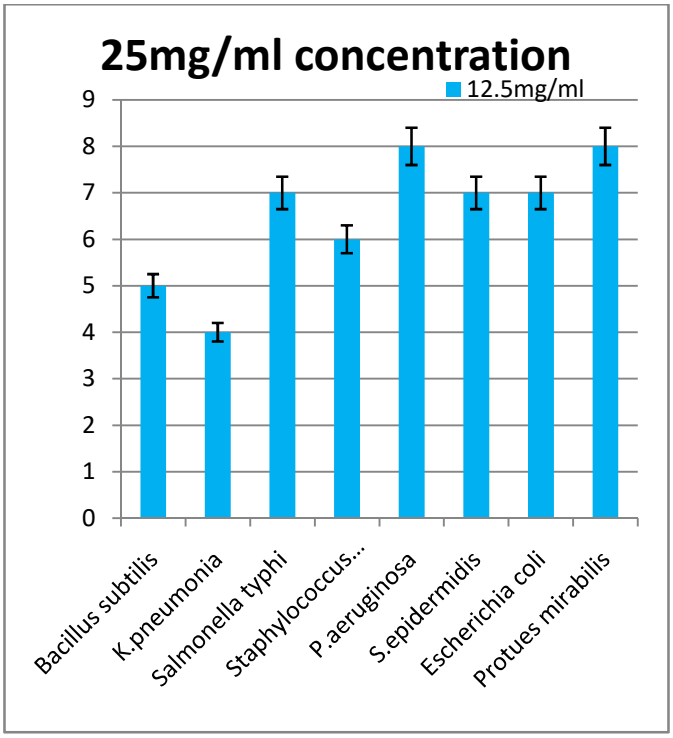


Chart 3.4. Inhibition zone of *Ricinodendron heudelotii* essential oil (Seed) using N- hexane solvent against multidrug resistance isolates

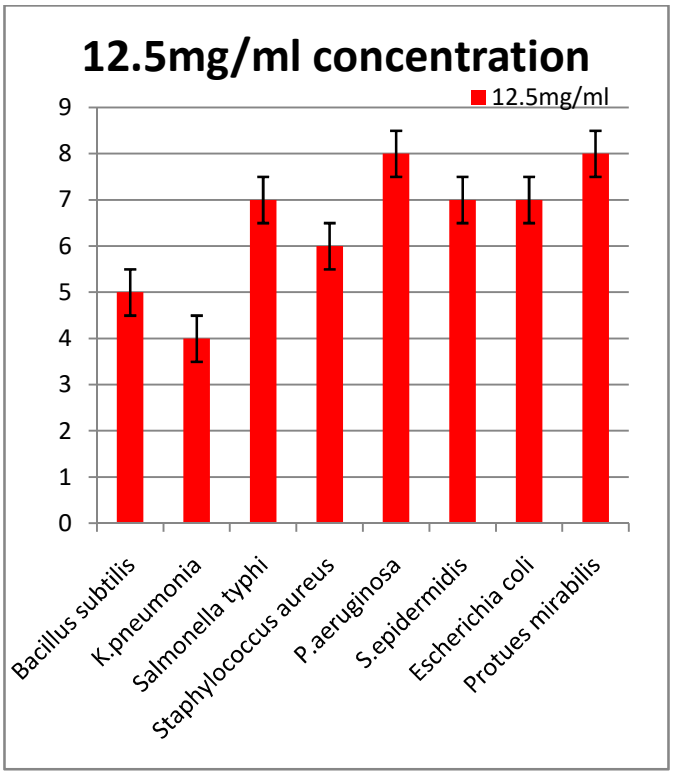


Chart 3.5 . Inhibition zone of *Ricinodendron heudelotii* essential oil (Seed) using ethyl acetate solvent against multidrug resistance isolates.

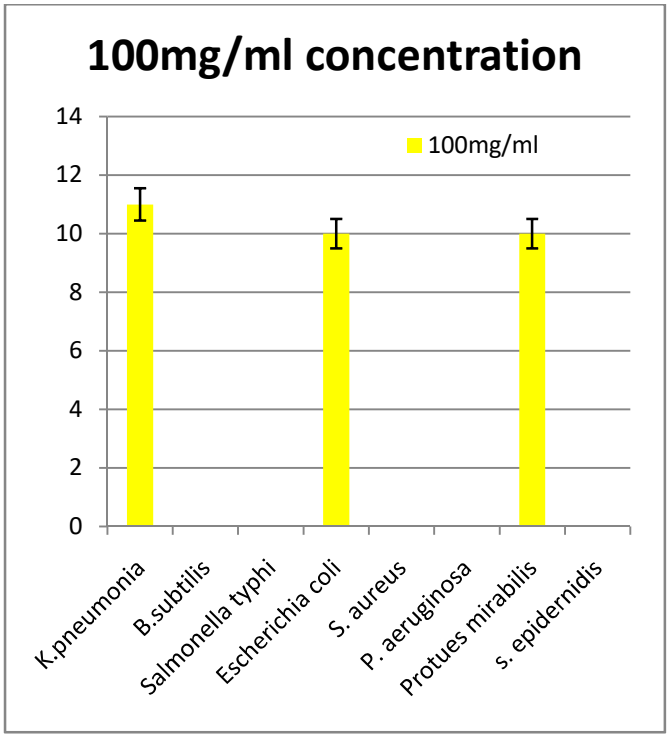


Chart 3.6 Inhibition zone of *Ricinodendron heudelotii* essential oil (Seed) using ethyl acetate solvent against multidrug resistance isolates.

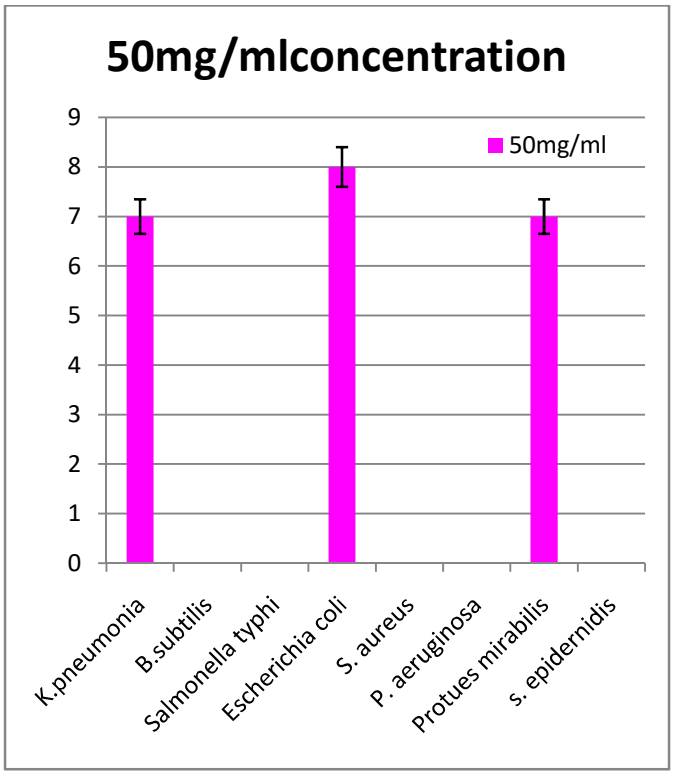


Chart 3.7 Inhibition zone of *Ricinodendron heudelotii* essential oil (Seed) using ethyl acetate solvent against multidrug resistance isolates.

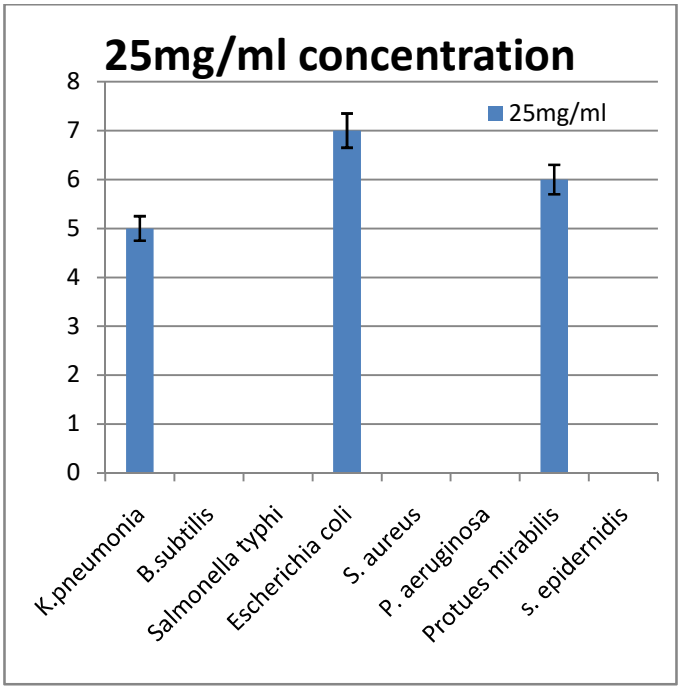


Chart 3.8. Inhibition zone of *Ricinodendron heudelotii* essential oil (Seed) using ethyl acetate solvent against multidrug resistance isolates.

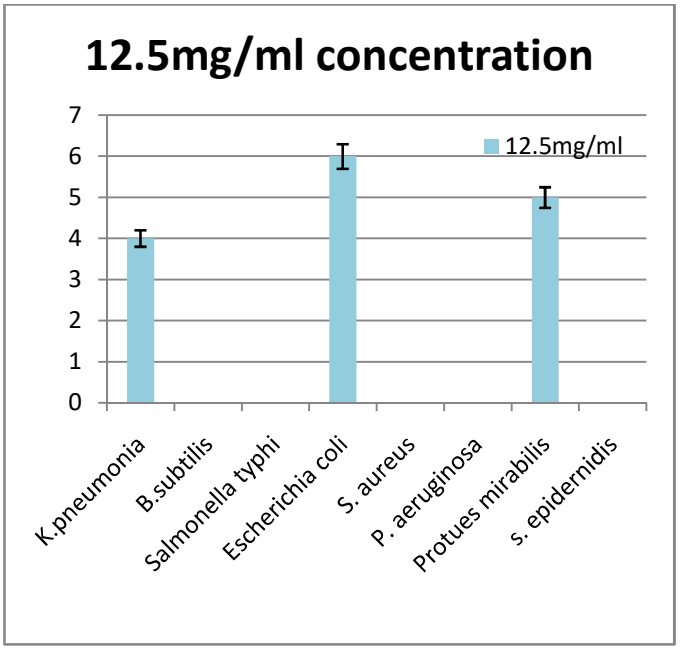


Chart 3.9 – Chart 3.12: Antibacterial activity of *Ricinodendron heudelotii* (Mull. Arg.) (stem bark) essential oil using N-hexane as solvent.

Indicate that the essential oil of *Ricinodendron huedelotii* (stem bark)using N-hexane as solvent expressed in mm with *Staphylococcus aureus*, showing the widest zone of inhibition (14.0mm) while the least zone of inhibition (3.0mm) was recorded for *Bacillus subtilis* . The essential oil of *Ricinodendron huedelotii* (stem bark) extracted using N-hexane as solvent act as a strong antibacterial agent against *Staphylococcus aureus*, *Salmonella typhii*, *Bacillus subtilis*, *Klebsiella pneumonia, Escherichia coli* and *Proteus mirabilis* in-vitro. The oil showed activity against both gram positive and gram negative bacteria. On the average, antibacterial activity of the essential oil was more pronounced on gram positive bacteria (mean zone of inhibition: 12.0mm) than gram negative (11.0mm).

Chart 3.13 - Chart 3.16: Antibacterial activity of *Ricinodendron heudelotii* (Mull. Arg.) (stem bark) essential oil using ethyl acetate as solvent.

shows that the essential oil of *Ricinodendron huedelotii* (stem bark) extracted using ethyl acetate as solvent were found to be active against only two gram positive bacteria (*Klebsiella pneumonia* and *Escherichia coli*) and gram negative bacteria(*Proteus mirabilis*) as shown by the zone of inhibition below. The *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella epidermidis, Salmonella typhi* and *Bacillus subtilis* are resistance to the oil since no zone of inhibition were observed. On the average,antibacterial activity of essential oil of *Ricinodendron huedelotii* (stem bark) using ethyl acetate as solvent for the extraction was more pronounced on gram negative bacteria(zone of inhibition:12.0mm) than gram positive bacterial(11.0mm).

Chart 3.9. Measurement of zone of inhibition of *Ricinodendron heudelotii* essential oil (Stem bark) using N-hexane solvent against multidrug resistance isolates.

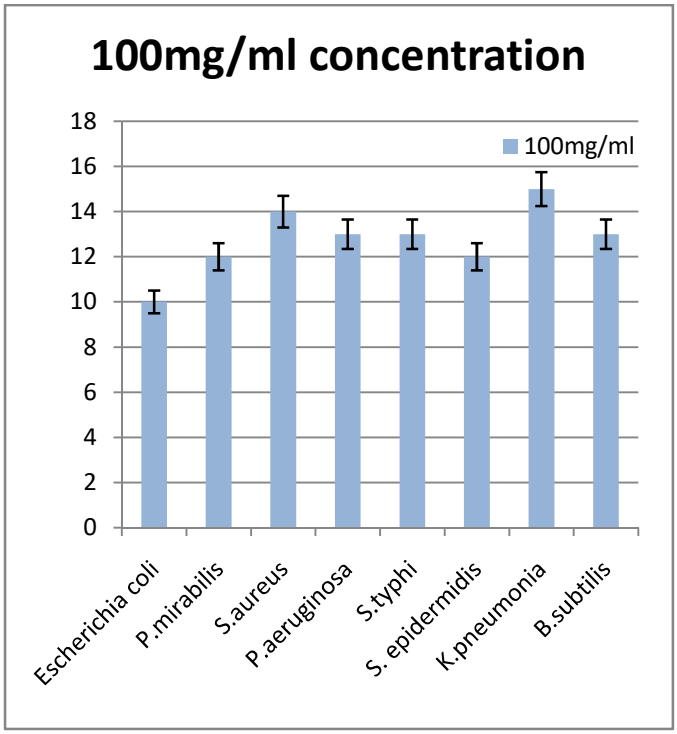


Chart 3.10. Zone of inhibition of *Ricinodendron heudelotii* essential oil (Stem bark) using N- hexane solvent against multidrug resistance isolates.

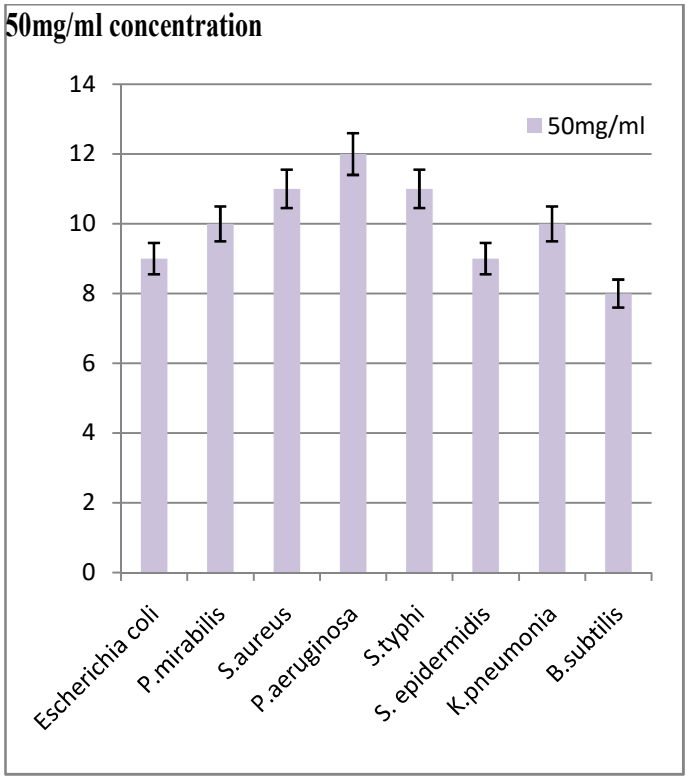


Chart 3.11. Zone of inhibition of *Ricinodendron heudelotii* essential oil (Stem bark) using N- hexane solvent against multidrug resistance isolates.

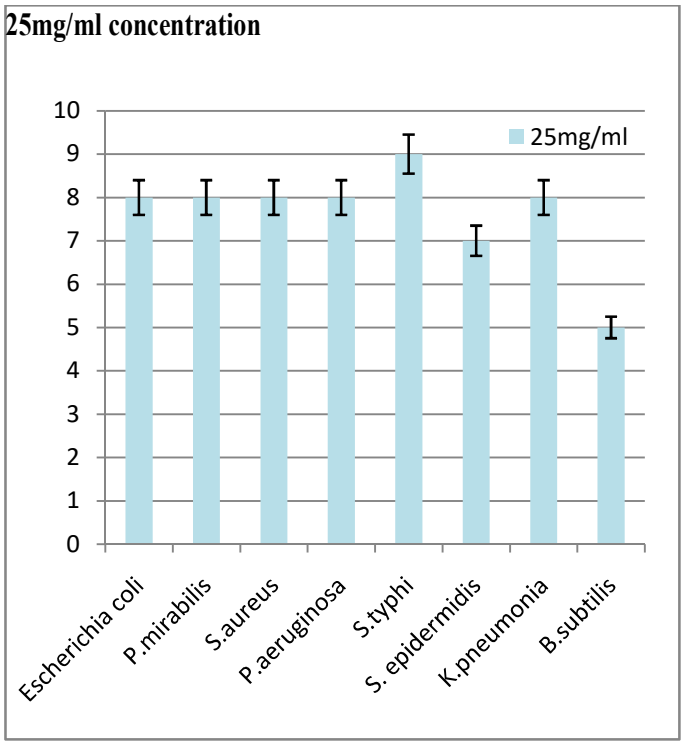


Chart 3.12. Zone of inhibition of *Ricinodendron heudelotii* essential oil (Stem bark) using N- hexane solvent against multidrug resistance isolates.

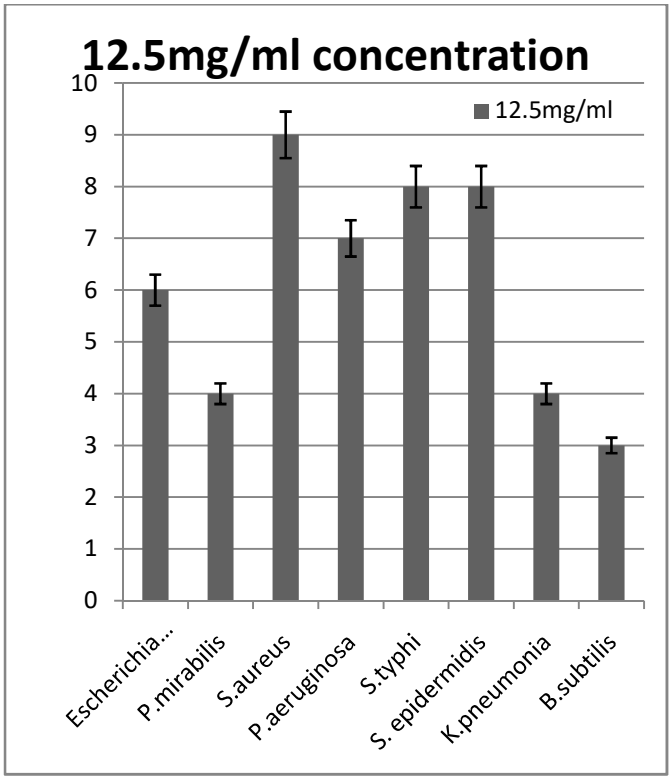


Chart 3.13. Zone of inhibition of *Ricinodendron heudelotii* essential oil (Stem bark) using ethyl acetate solvent against multidrug resistance isolates.

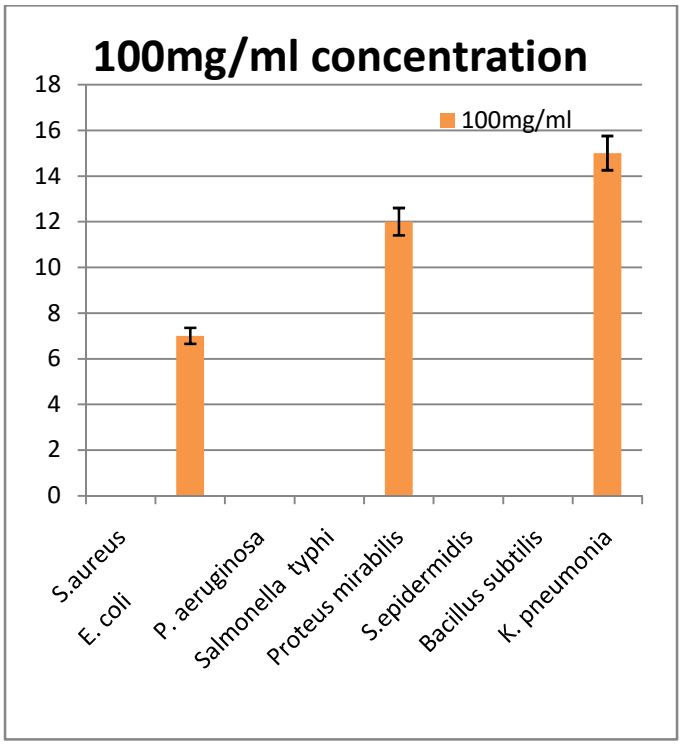


Chart 3.14. Zone of inhibition of *Ricinodendron heudelotii* essential oil (Stem bark) using ethyl acetate solvent against multidrug resistance isolates.

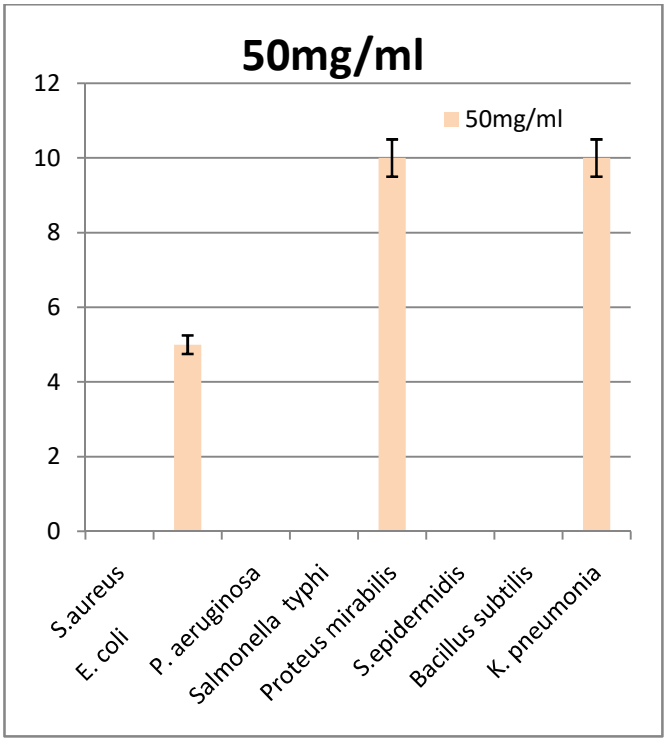


Chart 3.15. Zone of inhibition of *Ricinodendron heudelotii* essential oil (Stem bark) using ethyl acetate solvent against multidrug resistance isolates.

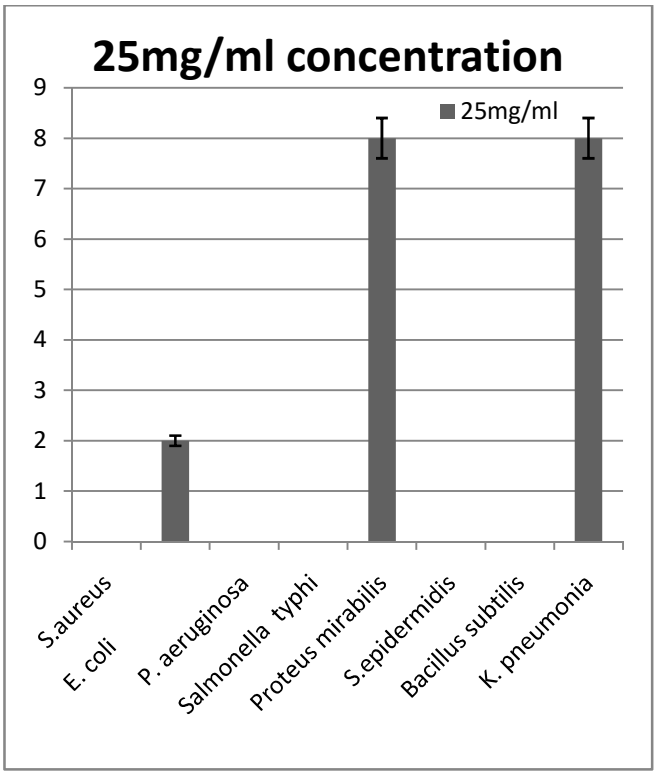


Chart 3.16. zone of inhibition Of *Ricinodendron heudelotii* essential oil (Stem bark) using ethyl acetate solvent against multidrug resistance isolates.

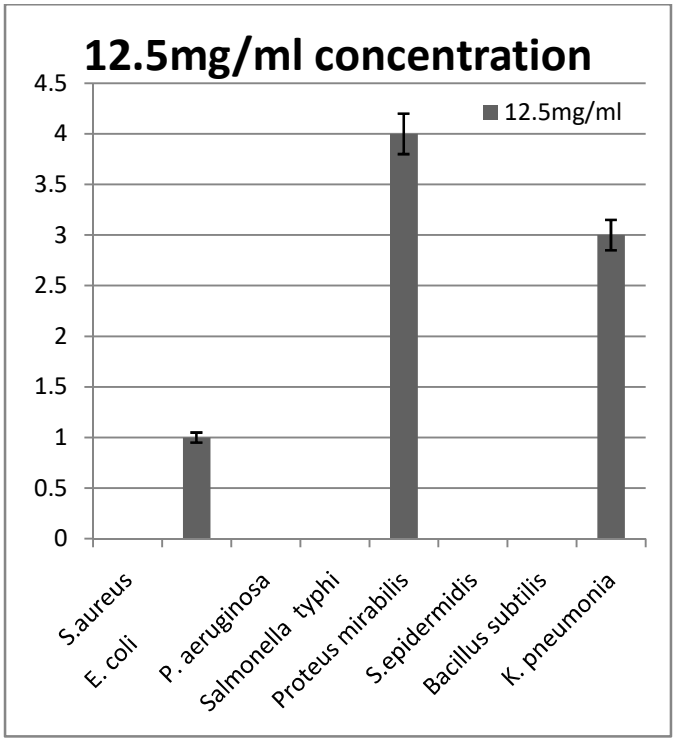


Table 3.1Qualitative Phytochemical Analysis of *Ricinodendron heudelotii* (Mull. Arg.)

	Ricinodendron heudelotii (Mull. Arg.)
Alkaloid	+ve
Glycoside	+ve
Steroid	+ve
Anthraquin	**ND**
Phenol	+ve
Tannins	+ve
Saponin	+ve
Flavonoid	+ve
Pyrrolizidine alkaloid	+ve
Reducing agent	+ve
Terpenoid	+ve
Volatile oil	+ve
Cardiac glycoside	+ve

KEY

+ve means positive

-ve means negative and

ND means not detected

Table 3.2: Quantitative Phytochemical Analysis of *Ricinodendron heudelotii (Mull. Arg.)*

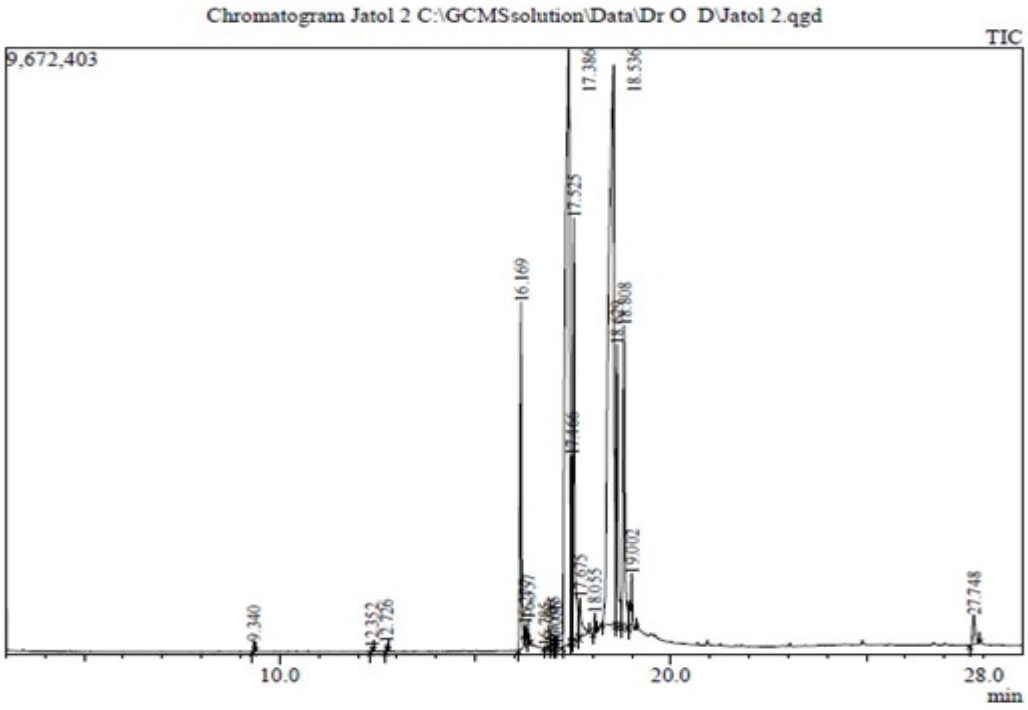
Ricinodendron heudelotii (Mull. Arg.)			
	Methanol	Ethanol	Ethyl acetate
Alkaloid	17.30	3.50	4.03
Glycoside	17.27	3.55	10.14
Steroid	15.56	4.25	2.31
Anthraquin	15.71	2.47	6.09
Phenol	12.36	3.48	8.09
Tannins	12.42	3.44	6.70
Saponin	1.25	3.21	5.83
Flavonoid	1.30	3.50	4.02
Pyrrrolizidine alkaloid	1.87	3.55	10.14
Reducing agent	17.30	4.25	2.31
Terpenoid	17.27	2.47	6.09
Volatile oil	15.65	3.48	8.09
Cardiac glycoside	15.71	3.44	6.70

Table 3.3 Gas Chromatograph - Mass Spectrophotometry profile of *Ricinodendron heudelotii* (Mull. Arg.) Seed Using N-Hexane as Solvent.

The seed of *Ricinodendron heudelotii* gave a yellowish oil. The constituents identified by GC/MS analysis, their compound name, structural formula retention time, base peak, molecular number, and height percentage are summarised in table below, in table 3.5, seven components were identified (100%). The oil was dominated by hydrocarborns, characterized by a high percentage of Oxacycloheptadec 8-en-one (20.48%), other major components were Heptadecanoic acid ($C_{19}H_{38}O_2$) with the retention time of 17.525, 1.37% height, 88.05 base peak and 298 molecular weight. Gamolenic acid ($C_{18}H_{30}O_3$) with the retention time of 18.810, 1.84% height, 79.05 base peak and 278 molecular weight.

Appreciable amount were: Pentadecanoic acid ($C_{15}H_{30}O_2$) with the retention time of 16.170, 11.95% height, 43.05 base peak and 242 molecular weight. Oxycycloheptadec 8-en-one ($C_{16}H_{28}O_2$) with the retention time of 17.385, 20.48% height, 67.05 base peak and 252 molecular weight. n-propyl 9, 12-Octadecadienoic acid ($C_{18}H_{36}O_2$) with the retention time of 17.525, 14.55% height, 43.05 base peak and 284 molecular weight. Octadecetrieonoic acid, ethyl ester ($C_{20}H_{34}O_2$) with the retention time of 18.630, 10.30% height, 79.05 base peak and 306 molecular weight. Oxacycloheptadec 8-en-one show a height percentage of (20.48%) while gama-linolenic acid, methyl ester showing the high retention time of 19.00 given a height percentage of (1.00%), 79.05 base peak and 292 molecular weight.

3.2.1 Spectra of *Ricinodendron heudelotii* (seed) using N-hexane as solvent for the extraction of the oil.



Gas chromatography analysis spectra for N-hexane extracts of *Ricinodendron heudelotii (seed)*.

Table 3.3: Chemical composition of *Ricinodendron heudelotii* seed essential oil using n-hexane as solvent for extraction.

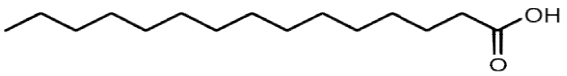
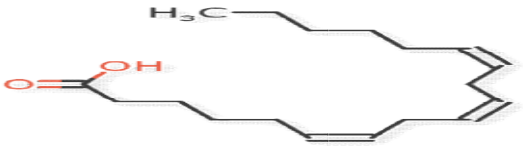
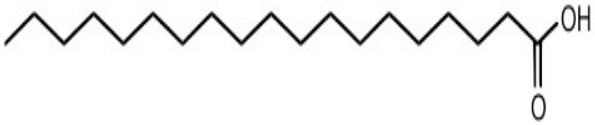
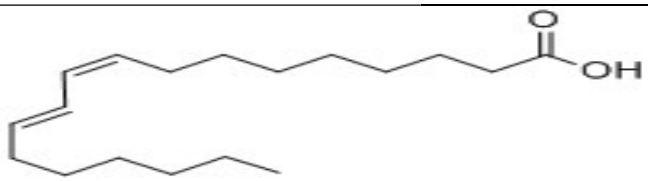
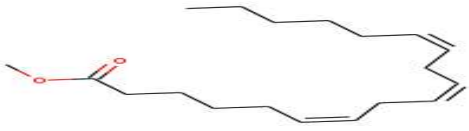
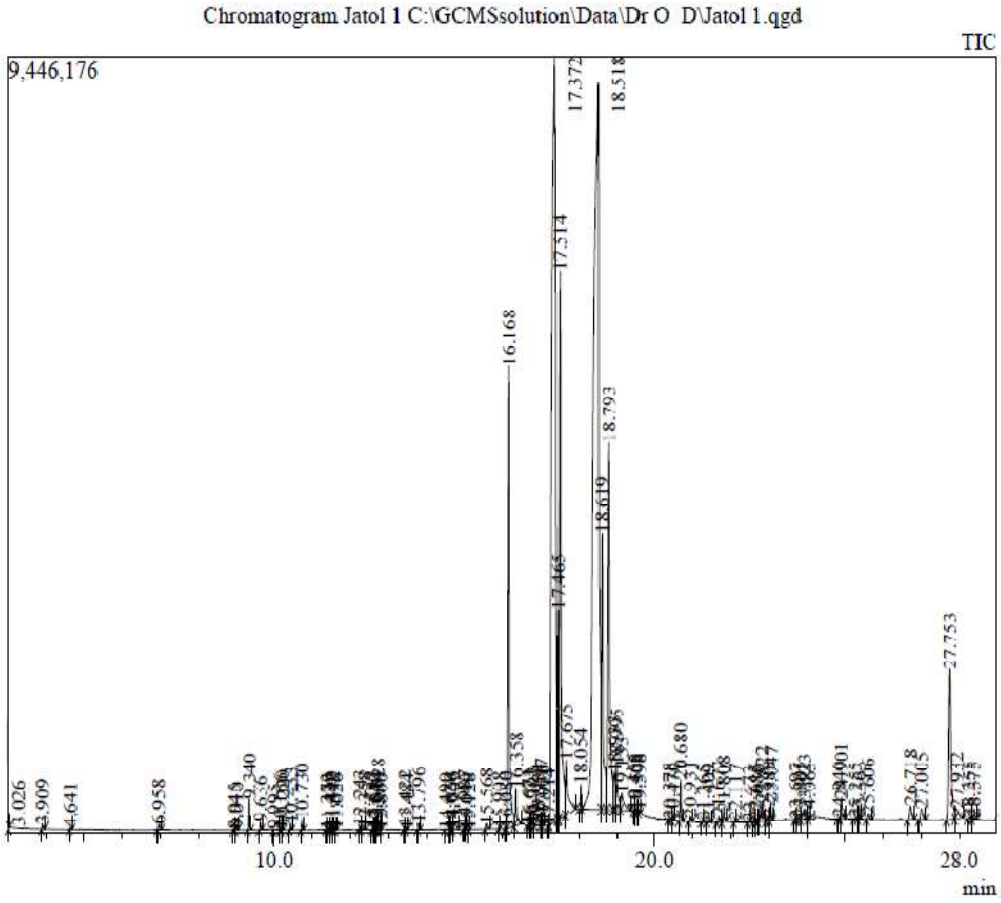
Structural name	Molecular Weight	Structural formular	Retention time	Base peak	Height%	Structures
Pentadecanoic acid	242	$C_{15}H_{30}O_2$	16.170	43.05	11.95	
Oxacycloheptadec 8 -en -one	252	$C_{16}H_{28}O_2$	17.385	67.05	20.48	
n-propyl9,12-octadecadienoate	322	$C_{21}H_{38}O_2$	17.465	81.10	6.40	
Octadecanoic acid	284	$C_{18}H_{36}O_2$	17.525	43.05	14.55	
Heptadecoic acid ethyl ester	298	$C_{19}H_{38}O_2$	17.675	88.05	1.37	
8,11,14-Eicosatrienoic acid	306	$C_{20}H_{34}O_2$	18.535	79.05	19.23	
9,12,15-Octadecatrienoic acid , ethyl ester	306	$C_{20}H_{34}O_2$	18.630	79.05	10.30	
Gamolenic acid	278	$C_{18}H_{30}O_2$	18.810	79.05	1.84	

Table 3.4 Gas Chromatography - Mass Spectrophotometry profile of *Ricinodendron heudelotii* (Muh.Arg) Seed using ethyl acetate as Solvent.

The constituents identified by GC/MS analysis are summarised in table below, in table 3.6, 10 components were identified (100%). The oil was dominated by hydrocarborns: Nonadeinoic acid ($C_{21}H_{42}O_2$) with the retention time of 17.675, 1.34% height, 88.05 base peak and 284 molecular weight. 9,12,15-Octadecatrienoic acid, ethyl acetate ($C_{20}H_{34}O_2$) with the retention time of 18.995, 1.09% height, 79.05 base peak and 306 molecular weight. Cholesterol ($C_{27}H_{46}O$) with the retention time of 27.755, 3.67% height, 43.05 base peak and 386 molecular weight. Disooctyl phthalate ($C_{24}H_{38}O_4$) with the retention time of 20.680, 1.01% height, 149 base peak and 390 molecular weight.

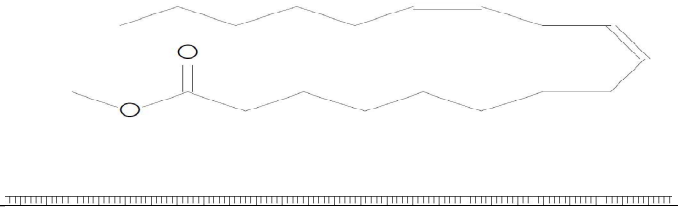
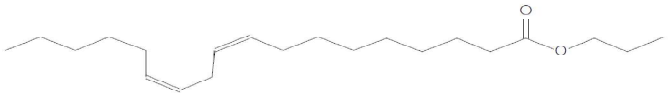
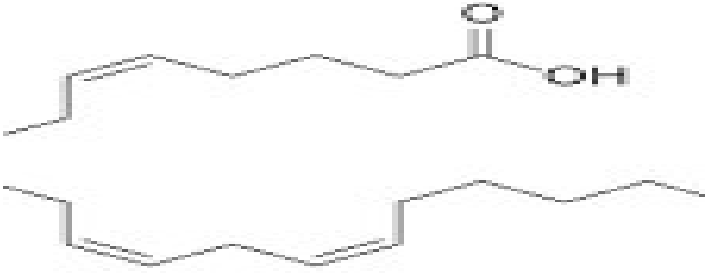
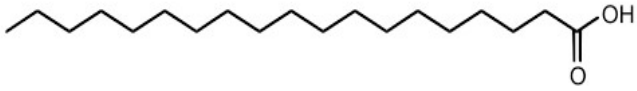
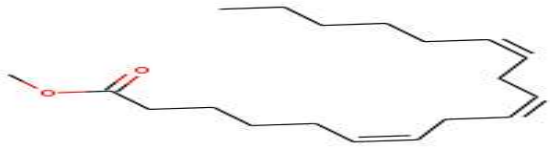
Appreciable amount were: Octadecanoic acid ($C_{18}H_{36}O_2$) with the retention time of 17.515, 13.35% height, 43.05 base peak and 284 molecular weight. Ascorbic acid ($C_{20}H_{46}$) with the retention time of 16.080, 11.27% % height, 73.05 base peak and 652 molecular weight. Gamolenic acid ($C_{18}H_{30}O_2$) with the retention time of 18.515, 17.74% height, 79.05 base peak and 278 molecular weight. Linolenic acid, methyl ester ($C_{19}H_{32}O_2$) with the retention time of 18.620, 6.74 % height, 79.05 base peak and 292 molecular weight. n-propyl 9,12-octadecadienoic acid ($C_{21}H_{38}O_2$) with the retention time of 17.465, 5.12 % height, 81.10 base peak and 322 molecular weight. 8,11,14-Eicosatrieonoic acid ($C_{20}H_{34}O_2$) with the retention time of 18.795, 8.94% height, 79.05 base peak and 306 molecular weight. 9, 12-Octadecadienoate acid ($C_{18}H_{32}O_2$) with the retention time of 17.370, 18.68% height, 67.05 base peak and 280 molecular weight while alpha Amyrin ($C_{30}H_{50}O$) showing the high retention time of 28.375 given a height percentage of 0.05%, 218.20 base peak and 426 molecular weight.

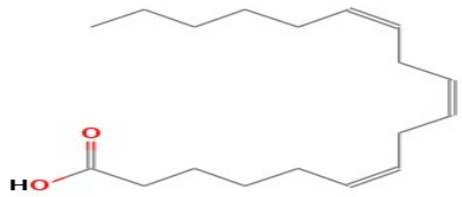
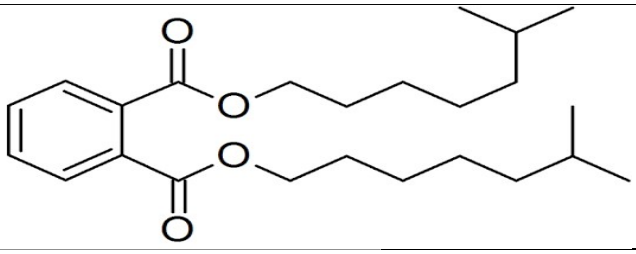
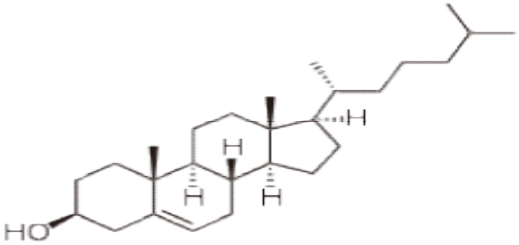
3.2.2 Spectra of *Ricinodendron heudelotii* (seed) using ethyl acetate as solvent for the extraction of the oil.



Gas chromatography analysis spectra for ethyl acetate extracts of *Ricinodendron heudelotii (see)*

Table 3.4: chemical composition of *Ricinodendron heudelotii* (Mull. Arg.) seed essential oil extracted by ethyl acetate

Structural name	Molecular Weight	Structural formular	Retention time	Base peak	Height%	Structures
Pentadecanoic acid	242	$C_{15}H_{30}O_2$	16.170	43.05	11.27	
9,12-Octadecadienoic acid	280	$C_{18}H_{32}O_2$	17.370	67.05	18.68	
n-propyl 9,12-Octa decadienoate	322	$C_{21}H_{38}O_2$	17.465	81.10	5.12	
Octadecenoic acid	284	$C_{18}H_{36}O_2$	17.515	43.05	13.35	
Nonadecanoic acid ethyl ester	326	$C_{21}H_{42}O_2$	17.675	88.05	1.34	
Gamolenic acid	278	$C_{18}H_{32}O_2$	18.515	79.05	6.74	

Gamma-Linolenicacid, methyl ester	292	$C_{19}H_{32}O_2$	18.620	79.05	6.74	
8,11,14-Eicosatrinoic acid	306	$C_{20}H_{34}O_2$	18.795	79.05	8.94	
Diisooctyl phthalate	390	$C_{24}H_{38}O_4$	20.680	149.00	1.01	
Cholesterol	386	$C_{27}H_{48}O$	27.755	43.05	3.67	

4.0 DISCUSSION AND CONCLUSION

4.1 Discussion

The purpose research work is to evaluate the antibacterial activity, gas chromatography-mass spectrophotometry profile and secondary metabolite (phytochemical) analysis of essential oils derived from *Ricinodendron heudelotii* (Mull. Arg.) seed and stem bark using N-hexane and ethyl acetate as the extracting solvents. In the emergence of bacterial resistance to antibacterial agents, more effort is being made to find alternative antimicrobial components to combat the resistance organisms. This research revealed that *Ricinodendron heudelotii* seed and stem bark essential oils exhibited strong antimicrobial activity against the selected multiple resistance clinical isolates. (31,32). The current study shows the role of essential oil of *Ricinodendron huedelotii* (seed and stem bark) using two distinct solvent (N-hexane and ethyl acetate) as a strong antibacterial agent against *Bacillus subtilis, Klebsiella pneumonia, Staphylococcal aureus, Salmonella epidermidis, Escherichia coli, Salmonella typii* and *Proteus mirabilis*, and may be considered as a useful lead in the search of new drugs (39,40).

Essential oils are potential source of antimicrobial compounds especially against bacterial. In-vitro studies in this work, shows that essential oils inhibits antibacterial growth against various multiple resistant clinical organisms, but their effectiveness varied. In table 1.1, Antibacterial activity of Essential oil was more effective on Gram positive bacterial (mean zone of inhibition 13.0mm) than Gram negative whose mean zone of inhibition is 11.0mm, due to complex structure of Gram-negative bacteria having a thick peptidoglycan layer of 2-3mm which is thinner in Gram-positive bacteria enabling hydrophobic molecules to easily penetrate the cells and act on both cell wall and the cytoplasm (33, 34,35). The current study shows the role of essential oil of N-hexane extract from *Ricinodendron heudelotii* (Seed and stem bark) as a strong antibacterial agent against multidrug resistance clinical isolates, this can be consider as a useful lead in the search of new drugs.

The essential oil of *Ricinodendron huedelotii* (Mull. Arg.) Seed and Stem bark were found more active on *Klebsiella pneumonia* and two other Gram-negative bacteria (*Salmonella typhi* and *Proteus mirabilis*) as shown by the inhibition zones in chart 3.2. On the average, antibacterial activity of essential oil were very active against Gram positive bacteria (zone of inhibition 11.00mm) because the bacterial cell wall of Gram positive bacterial allows hydrophobic molecules to easily penetrate the cells thus, the susceptibility of Gram –positive bacteria. The antibacterial activity of both parts of the plant summarizes the microbial growth inhibition by the essential oil of *Ricinodendron heudelotii* seed and stem bark using N-hexane as extracting solvent which shows good antibacterial activities against multiple resistance clinical isolates. Essential oils from *Ricinodendron heudelotii* seed exhibit more bacterial activity against most bacterial at higher concentration, which reveals the susceptibility of both Gram positive and Gram negative bacteria at higher concentration. A more significant zones of inhibition was seen with a higher essential oil concentration. At low concentration, a very limited inhibitory zones was observed (36,37).

Comparing the efficacy of the essential oil of *Ricinodendron heudelotii,* using agar well diffusion method. *Ricinodendron heudelotii* seed using n-hexane as the extracting solvents has the highest inhibition zone (15.0mm) at the highest concentration of 100mg/ml against *Bacillus subtilis* and *Pseudomonas aeruginosa* < inhibition zone (14.0) against *Klebsiella pneumonia* (chart 3.1-3.4).

The effectiveness of *Ricinodendron heudelotii* seed using N-hexane as extracting solvent, due to its high content of ascorbic acid,which may be used to stimulate the production of white blood cell primarily neutrophile attacks foreign antigens and boost production of antibodies (38). Essential oil from *Ricinodendron heudelotii* (stem bark using N-hexane as solvent) was also quiet effective with the highest inhibition zone of (15.0mm)against *Klebsiellla pneumonia* at 100mg/ml, followed by (14.0mm and 13.0mm) against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively while at a concentration of 12.5mg/ml shows the least inhibition zone of (3.0mm) against *Bacillus subtilis* (chart 3.9-3.12). Essential oil from *Ricinodendron heudelotii* seed using N-hexane has the higher antibacterial activity against the clinical isolates compared to essential oil from *Ricinodendron heudelotii* stem bark using ethyl acetate as solvent. *Ricinodendron heudelotii* seed using N-hexane as solvent has a higher zone of inhibition (15.0mm) against *Bacillus subtilis* and *Pseudomonas aeruginosa* and lowest inhibition zone (1.00mm) against *Klebsiella pneumonia* at 12.5mg/ml (chart 3.1 - chart 3.16).

Secondary metabolite (Phytochemical) screening exhibits most of the natural products.The qualitative analysis of *Ricinodendron heudelotii* (Mull. Arg.)using different solvent were expressed during this research work. It was observed that all secondary metabolite were present except anthraquinone that was not detected. Alkaloids, cardiac glycosides, steroids, phenol, tannins, saponins, reducing sugar, flavonoid, pyrrolizidine alkaloid, terpenoid and volatile oil were all present (table 3.1).

Alkaloids are medically useful and have been reported to possess antimicrobial, antioxidant activity (41,42). Flavonoid are hydroxylated phenolic substances known to be synthesized by plant in response to microbial infection and it should not be surprising that they have been found invitro to be effective antimicrobial substances against a wide array of microorganism (43). The antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell (44,45). Saponin has also been reported to have anti-inflammatory, cardiac depressant and hyper-cholesterolemic. Saponin and steroid also have relationship with sex hormones like oxytocin which regulates the onset of labour in pregnant women and subsequent release of milk. Taninin are used as antidiarrheal, saponin are glycoside of triterpenes, steroid alkaloid found in plant are useful for lowering cholesterol and displays analgestic properties (46).

The quantitative secondary metabolite screening of *Ricinodendron heudelotii* (Mull. Arg.) using methanol as solvent. Alkaloids and reducing sugar were shown to be present in the largest quantity with the same value of 17.30 and saponin was found to be the least present with 1.25. Using ethanol as solvent, reducing sugar and steroid were shown to be present in the largest quantity with the same value of 4.25, anthraquinone and terpens were found to be the least present with the same value of 2.47.Using ethyl acetate as solvent, glycoside and pyrrolizidin alkaloid were shown to be present in the largest quantity with the same value of 10.14, steroid and reducing sugar were found to be the least present with the same value of 2.31.

Gas chromatography and mass spectrophotometer of essential oil from *Ricinodendron heudelotii* (Mull. Arg.) Seed using N-hexane as solvent, reveals 20 active compounds with different isomers present in *Ricinodendron heudelotii* (seed) oil as shown in Table 3.5.The total percentage yield of chemical compound present equal to 100%. GC-MS analysis revealed oxacycloheptadec 8-en-one, Eicosatrienoic acid and octadecanoic acid to be the major constituent of *Ricinodendron heudelotii* (seed using N-hexane as solvent) (47,48). Oxacycloheptadec 8-en-one shows a height percentage of (20.48%) with a retention time of 17.385 while linolenic acid, methyl ester having a high retention time of 19.00 gives a height percentage of (1.00%) with the height percentage of oxacycloheptadec 8-en-one,. Heptadecoic acid has it role as a mammalian metabolite while propyl 9,12-octadecadienoate a fatty acid resulted from the condensation of the hydroxyl group of propanol has a bacterial metabolites (49). Octadecanoic acid is used as food additive which is free fatty acid, which can also be absorbed in the regular diet (50,51) while Eicosatrienoic acid ia rare polyunsaturated fatty acid off the omega 3-series which appear to act as dual inhibitor of cyclooxygenase and lipoxygenase pathway (52).

9,12,15-octadecatrienoic acid,ethyl ester is metabolized and converted to conjugated linolenic acid that has shown the

potential as a tumor growth suppressor in colon cancer and it induce apoptosis (53). Gamolenic acid maintain youthful skin, working hormones an efficient metabolism. In the skin, gamolenic acid promotes hydration and elasticity inside and outside(cracking skin, dry hair may indicate lack of gamolenic acid), this acid also make a certain kind of prostaglandin (a fat that work like hormones) to activate the metabolism through this massaging network and gamolenic acid potentiates fat loss and boosts energy (54,55,56) while cholesterol serves as a precursor for the biosynthesis of steroid hormones, bile acid and vitamine D which composed about 30% of all cell membrane fluidity over the range of physiological temperatures(56,57).

Gas chromatography and mass spectrophotometer ethyl acetate extract of *Ricinodendron heudelotii* seed essential oil reveals 91 active compound with difference isomers present *Ricinodendron heudelotii* seed as shown in Table (3.6) the total percentage yield of chemical compound presents equals to 100%.

GC-MS revealed 9,12-Octadecadenoi acid(18.68%), ascorbic acid (11.29%), propyl 9,12- octadecadenoic acid (5.12%), octadecadenoic acid(13.38%), gamolenic acid (17.74%)and linolenic acid methyl ester(6.74%) as the major constituent of *Ricinodendron heudelotii* seed using ethyl acetate as solvent. 9,12-Octadecadenoi acid shows a height percentage of (18.68%) with retention time of 17.370 while alpha-amyrin showing the high retention time of 38.375 gives a height percentage of (0.05%). With the height percentage of 9,12-Octadecadenoi acid, it is regarded as the major constituent of ethyl acetate extract of *Ricinodendron heudelotii* seed(58).

Eicosatrienoic acid also known as dihomogamma-linolenic acid can be converted into prostaglandin that inhibits platelets aggregation and also exerts a vasodilatory effects, diisooctyl phthalate stabilizes membranes of red blood cells enabling blood product storage and ascorbic acid play a definitive role in treating scurvy also for the prevention and treatment of various diseases and it can also be considered as the most effective and safe medicines needed in a health system (59,60).

The result shows that essential oils varied significantly in their antibacterial potential. These differences may be attributed to differences in nature and concentration of chemical constituent in the different plant parts(61). Major active constituent present in the seed and stem bark of *Ricinodendron heudelotii* essential oils, using N- hexane and ethyl acetate as solvent are indicated in (chart 3.1-3.16).

4.2 Conclusion

This study indicates that essential oils serve as an important source of antibacterial compounds that can provide renewable source of useful antibacterial drug against bacterial infections in human. The results of this study present essential oil as good antibacterial agent to combat pathogenic microorganisms. The essential oil from the seed of *Ricinodendron heudelotii* (Mull. Arg.) and from the stem bark of *Ricinodendron heudelotii* (Mull. Arg.) showed varying degrees of antibacterial activity against selected clinical isolates . From the study, it can be inferred that essential oil extracted using N-hexane as solvent from the seed and stem bark of *Ricinodendron heudelotii* (Mull. Arg.) Shows significant growth inhibition activity on the selected multiple resistance clinical isolates. The efficacy of seed and stem bark of *Ricinodendron heudelotii* (Mull. Arg.),against these microorganisms provides a scientific ground for the application of the herb in the prevention and treatment of bacteria infections especially the multiple resistance bacterial which have the ability of developing resistance to antibiotics. The general usefulness of essential oil cannot be over emphasised as it is more beneficial than synthetic drugs.

4.3 Recommendation

- Research can be carried out on essential oils greatest potential use as a food preservative since they have been known to inhibit bacteria, yeast and fungi.
- Further studies could also be carried out on the possible drugs that can be derived from *Ricinodendron heudelotii* (Mull. Arg.).
- Further studies could be carried out to determine the shelf life of essential oil and to assess their rate of deterioration if it is use as medicine.

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