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Quantitative phytochemical screening of four Nigerian medicinal plants and their biological activities againts some clinical pathogens

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Abstract
Curcuma longa, phoenix dactylifera, Beta vulgaris and Zimgiber zerumber possesses numerous medicinal values which include their antidiabetic, anti-inflammatory, anticancer, antimicrobial as well asi-obesity. Hexanolic extracts of the plants were subjected to quantitative phytochemical screening and tested for antimicrobial activities (Sparfloxacin, Ciprofloxacin and fluconazole) were used as standard drugs. Twenty (20) bioactive secondary metabolites were quantified and detected in the samples. Phytate recorded the highest concentrations (43.536, 41.836, 42.28 and 45.231 µg/mol) while sapogenin recorded the lowest concentrations (0.873, 0.786, 0.236 and 0.153 µg/mol) for Curcuma longa, phoenix dactylifera, Beta vulgaris and Zimgiber zerumber respectively. The pathogens used in this study were Pseudomonas aeruginosa, Staphylococcus aereus, Salmonella entrerica, Citrobacter murliniae, Bacillus licheniformis, Micrococcus roseus, Bacillus subtilis, Salmonella typhi, Protus Mirabilis, Candida albicans, Candida krusei, Candida tropicalis, Aspergillus niger and Aspergillus fumigatus. All pathogens were confirmed sensitive to the plant extracts. This work has shown scientific evidences on the usefulness of the plants investigated and their uses in trado-medicines to ascertain its claims by ethno-medical practitioners as well as their ability to serve as antibiotic drug candidates.

Keywords: quantitative, phytochemical, Nigerian, pathogens

Introduction
The most significant area of traditional medicine has been characterised globally by herbal prescription. The traditional use of medicinal plants contributes to the dissemination of this knowledge and serves as a groundwork for scientific research seeking confirmation of such pharmacological activities. The study of medicinal plants is vital to endorse the suitable use of herbal medicine in order to decide their potentials as a source for new drugs (Silver *et al*., 2016, Deb *et al*., 2013) [1]. Due to the numerous diseases and the current model of treatment based on synthetic drugs which have caused harms to the human body system and also too expensive. Considering the economy of the citizenry in this country Nigeria today, the several diseases and lack of money for treatment, safe and sound treatment is needed to control the disease development and progression. In this regards, medicinal plants and its constituents have been used for diseases management. Since medicinal plants has proven to be effective, less expensive and have lesser side effective. Therefore, medicinal plants and their uses are presently on the increase, due to easy availability, accessibility affordability and promising efficacy comparable to the often high cost and adverse effects of standard synthetic drug agents (Smith-Hall *et. al*, 2014) [13]. They show many promising effects for various health problems such as stomachache, indigestion, joint pains, colds, coughs, cancer and heart diseases, and have also positive protecting activities such as anti-inflammatory, antioxidant, anticancer, etc. It is a known fact that plants produces some chemicals that protect them and also use them for protection against diseases in humans and other animals (Ejiofor and Nna, 2022) [2]. These chemicals are called phytochemicals and include alkaloids, tannins, steroids, flavonoids, triterpenes. Much have not been done on *Curcuma longa, phoenix dactylifera, Beta vulgaris* and *Zimgiber* zerumber parts chosen for this study. Thus, the authors of this present work decided to examine the phytoconstituents of these plants for possible pharmacological potential compounds and their biological activities against some clinical pathogens to ascertain the traditional medical claims for the drugs in ethno-medicine with the hope that the results of this work will provide scientific evidences further investigations.

Materials and Methods
Sample collection and Authentification
All four samples of *Curcuma longa, phoenix dactylifera, Beta vulgaris* and *Zimgiber zerumber* under investigation were collected from Ogoni in Rivers state during the dry season (January –March). They were authenticated at the herbarium in the Department of Forestry, Rivers State University, Port Harcourt, Nigeria. The specimen Voucher numbers are 2022 (2,6.8). The aerial parts of the plants were collected cleaned with water to remove sand and other dirt and allowed to air dried for two weeks.

Extraction
The dried samples of *Curcuma longa, phoenix dactylifera, Beta vulgaris* and *Zimgiber zerumber* were extracted with hexane using a soxhlet apparatus and the extracts were first concentrated with a rotary evaporator and then finally dried in an air oven at about 40^0C. The crude aqueous extracts were later subjected to solvent-solvent portioning between water and chloroform as described by Lohdip and Ugwu (2016) [5]. For phytochemicals analysis, 2g of each samples were weighed and transferred in four different test tubes and 15 ml ethanol and 10 ml of 50 % m/v potassium hydroxide

was added. Each sample's test tubes were given 60 minutes in a water bath set at 600C to respond. Following the reaction period, the test tube-contained reaction products were transferred to a separating funnel. 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water, and 5 ml of hexane were added to the funnel and used to effectively wash the tubes. These extracts were mixed and three times rinsed in 10ml of an aqueous 10% ethanol solution. The solvent was evaporated after the solution was dried with anhydrous sodium sulphate. 200 millilitres of the 1000 millilitres of pyridine used to solubilize each sample were transferred to a vial for analysis**.**

Quantification by GC-FID

On a BUCK M910 Gas Chromatography fitted with a flame ionisation detector, the phytochemical analysis was carried out. The column was a RESTEK 15 m MXT-1 (15 m × 250 um x 0.15 um). With a splitless injection of 2ul of sample at a linear velocity of 30cms1 and helium 5.0pa serving as the carrier gas with a flow rate of 40 min-1, the injector temperature was 2800C. The oven was turned on at a temperature of 2000C, heated to 3300C at a rate of 30C per minute, and maintained at this temperature for 5 minutes. At 3200C, the detector was in operation. The ratio between the area and mass of the internal standard and the area of the detected phytochemicals was used to identify the phytochemicals**.**

Antimicrobial activities

Antimicrobial activities of the hexanolic extract from each sample were performed using the Muliller Hinton agar method as described by Nna *et al*., (2018) [9]. The microorganisms used were *Pseudomonas aeruginosa*, *staphylococcus aureus*, *Salmonella enterica*, *Citrobacter murliniae*, *Bacillus lichenifermis*, *micrococcus rosers* and *Bacillus subtilis.* The test bacteria were standardized by using a sterile wire loop, to pick 3-5 pure cultures of the test microorganism and emulsified in 3-4ml of sterile physiological saline. The turbidity reading of the 0.5 McFarland standard was recorded as absorbance in a spectrophotometer at 540 nm, while the turbidities of the test organisms were adjusted to match the absorbance of the 0.5 McFarland standard at the same wavelength, using physiological saline. The antibacterial activities of the extracts against the test bacteria were evaluated by modified disc diffusion methods. Exactly 2µl of 0.5 McFarland standardized suspension of test bacteria ($1.5x10^8$ cfc ml^{-1}) were cultured onto the Mueller-Hinton plates by pour plate method. 50 µl of the extracts were used to impregnate the 6mm filter paper discs and placed on two portions of the agar plate. The inhibition zone diameters of the various plates were measured and recorded in millimetres. All experiments were done in triplicates. Negative controls were set up with sterile physiological saline and positive controls were set up using 50 µg/ml ciprofloxacin.

Results and Discussion

The results of the phytochemical screening of the hexanolic extract of the four plants investigated in this present study are summarised in Table 1. It revealed the presence of some bioactive secondary metabolites such as Kaempferol, ephedrine, anthocyanin, dihydrocyntisine, aphylidine, steroid, tannin, flavonones, ribalinidine, spartein, cardiac glycoside, phytate, oxalate, cyanogenin glycoside, catechin, proanthocyanidin, narigenin, flavone, sapogenin and ammodendrine. Research carried out on population consuming plant diet in phytate has showed that it lower incidence of cancer, which suggests that phytate has an anticarcinogenic effect. (Shamsuddin, 2002). So, these plants under investigation might have anticarcinogenic properties. Phytic acid is a natural antioxidant that is mainly found in grains, nuts, and seeds. Kaempferol is noted for reducing the risk of chronic diseases, especially cancer. It serve as an antioxidant defence for the body against free radicals and controls apoptosis, angiogenesis, inflammation, and metastasis. Ephedrine is used to treat myasthenia gravis, low blood pressure issues, nasal congestion, and breathing issues. For the treatment of bronchial asthma. Anthocyanin is widely known for its actions against diabetes, cancer, inflammation, infection, and obesity as well as the protection of cardiovascular disorders. Stiroids help inflammation-related disorders including asthma and eczema by reducing redness and swelling. Catechins are substances that can aid in controlling blood pressure, speed up weight reduction, and shield the brain from illness. As an antioxidant, it is also (Ejiofor and Nna, 2022) [2]. Calcium and oxalate combine to generate complexes (Calcium oxalate crystals). These oxalate crystals result in disorders like rickets and osteomalacia by preventing the body from absorbing and utilising calcium. On the other hand, flavonoids are often recognised for their cancer-fighting, antioxidant, anti-inflammatory, and antiviral capabilities. They have cardioprotective and neuroprotective properties**.**

Table 1: Quantitative Phytochemical Analysis of the Plants Under investigation in (µg/g)

Compounds	***Curcuma longa***	***phoenix dactylifera***	***Beta Vulgaris***	***Zimgiber Zerumber***
Kaempferol	4.321	2.7	2.393	2.41
Ephedrine	14.036	5.793	6.023	6.073
Anthocyanin	8.207	8.4	7.416	10.2321
Dihydrocyntisine	0.386	10.793	10.366	10.372
Aphylidine	7.873	12.45	12.97	12.973
Steroid	19.973	19.765	20.313	20.301
Tannin	23.363	21.553	22.73	22.734
Flavonones	25.96	24.58	25.653	25.68
Ribalinidine	24.704	34.12	34.576	40.231
Spartein	36.773	36.01	36.876	38.431
Cardiac Glycoside	9.35	39.9	15.46	20.61
Phytate	43.536	41.836	42.28	45.231
Oxalate	3.093	15.673	39.2	18.461
Cyanogenicglycoside	5.483	15.076	15.46	15.461
Catechin	31.653	10.413	4.113	4.071

Proanthocyannidin	34.15	31.363	33	33.03
Narigenin	38.64	26.841	27.533	27.531
Flavone	29.326	23.201	29.86	29.863
Sapogenin	0.873	0.786	0.236	0.153
Ammodendrine	20.616	24.206	44.17	31.431

Table 2: Zone on inhibition and antibacterial activities of *Beta vulgaris* leaves and against standard drugs

Test organism	**Zone of inhibition and activities (mm)**	**Standard Drugs (mm)**		
		Sparfloxacin	**Ciprofloxacin**	**Fluconazole**
Pseudomonas aeruginosa	S(23)	S(30)	R(0)	R(0)
Staphylococcus	S(16)	S(31)	R(0)	R(0)
Salmonella enterica	S(15)	S(27)	S(32)	R(0)
Citrobactermurli niae	S(20)	S(29)	R(0)	R(0)
Bacillus licheniformis	S(22)	R(0)	S(24)	R(0)
Micrococcus roseus	S(18)	R(0)	S(24)	R(0)
Bacillussubtilis	S(16)	S(32)	R(0)	R(0)
Salmonella typhi	S(27)	S(26)	S(20)	R(0)
Protus Mirabilis	S(31)	S(28)	S(23)	R(0)
Candida albicans	S(32)	R(0)	S(27)	S(21)
Candida krusei	S(21)	R(0)	S(27)	S(24)
Candida tropicalis	S(20)	S(25)	S(31)	S(20)

Table 3: Zone inhibition and antimicrobial activities of phoenix dactyliferal fruits

Test Bacteria	**Mean Zone inhibition**				**Sparfloxacin**	**Ciprofloxacin**	**Fluconazole**	**Mean**
	X	**Y**	**Z**	**Mean**	**X**	**Y**	**Z**	
Pseudomonas aeruginosa	27(S)	26(S)	24(S)	25.7	50(S)	98(S)	46(S)	48(S)
Staphylococcus aureus	20(S)	17(S)	19(S)	18.7	60(S)	64(S)	62(S)	62(S)
Salmonella enterica	17(S)	19(S)	22(S)	19.3	43(S)	44(S)	46(S)	44.3(S)
Citrobacter murliniae	23(S)	20(S)	18(S)	20.3	40(S)	37(S)	38(S)	38.3(S)
Bacillus licheniformis	24(S)	20(S)	22(S)	22	43(S)	47(S)	42(S)	44(S)
Micrococcus roseus	27(S)	21(S)	27(S)	25(S)	40(S)	37(S)	39(S)	38.7(S)
Bacillus subtilis	22(S)	20(S)	16(S)	19.3	43(S)	40(S)	38(S)	40.3(S)

Table 4: Preliminary antimicrobial of 1000mg/L susceptibility screening of the hexanolic extracts of *Zingiber zerumbet* (Bitter Ginger)

Test Bacteria	**Mean Zone of inhibition(mm)**				**Control/ drugs**			
	X	Y	Z	Mean	X	Y	Z	X
Pseudomonas aeruginosa	12	14	12	12.7(S)	50	48	46	48
Staphycoccus aureus	26	29	25	26.6(S)	60	64	62	62
Salmonella enterica	18	22	27	22.3(S)	43	44	46	44.3
Citrobacter murliniae	22	17	19	19.3(S)	40	37	38	38.3
Bacillus licheniformis	27	26	26	26.3(S)	43	47	42	44
Micrococcus roseus	22	20	18	20(S)	40	37	39	38.7
Bacillus substilis	27	25	27	26.3(S)	43	40	38	40.3

Table 5: Zone of inhibition of antimicrobial analysis of the ethylacetate extract of *Curcuma longa*

Test Organism	**Mean zone of inhibition**				
	X	**Y**	**Z**	**Mean**	**Control drug mean**
Psedomonas aereginosa	(S)22	(S)24	(S)26	24.67	48(S)
Staphylococcs aureus	(S)22	(S)28	(S)27	25.67	62.0(S)
Salmonella enterica	(S)10	(S)12	(S)10	10.67	44.3(S)
Candida albicans	(S)23	(S)25	(S)23	23.67	50.2(S)
Aspergillus niger	(S)18	(S)18	(S)21	19	42.3(S)
Aspergillus fumigatus	(S)33	(S)30	(S)20	27.67	40.1(S)

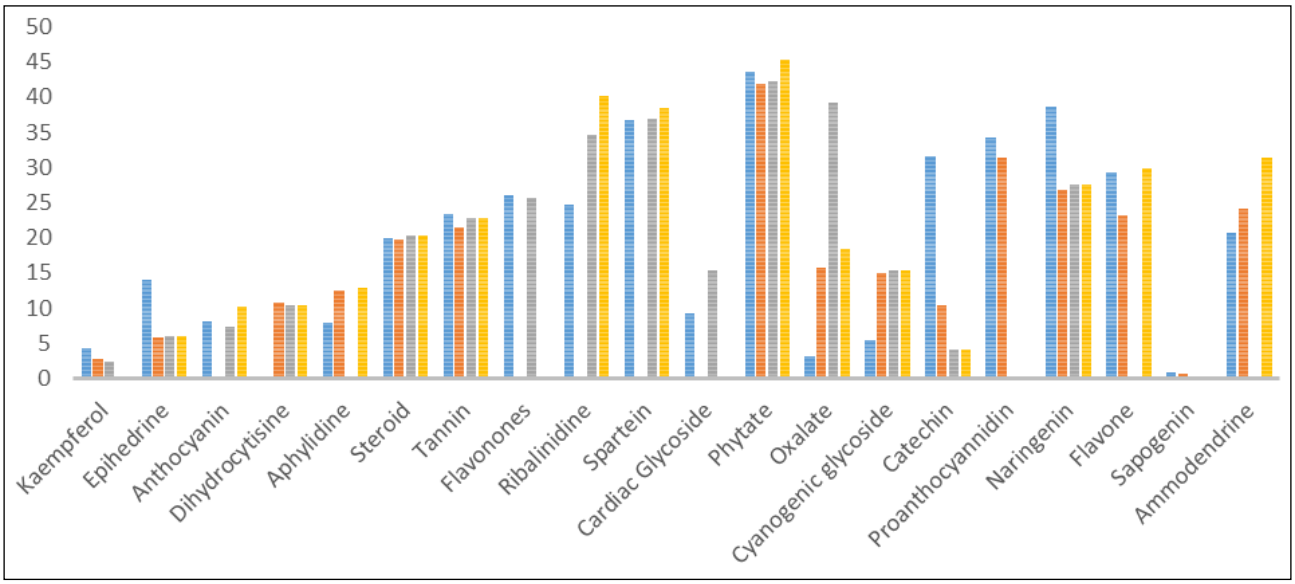

Fig 1: Quantitative phytochemical analysis of the plants under investigation in (µg/mol)

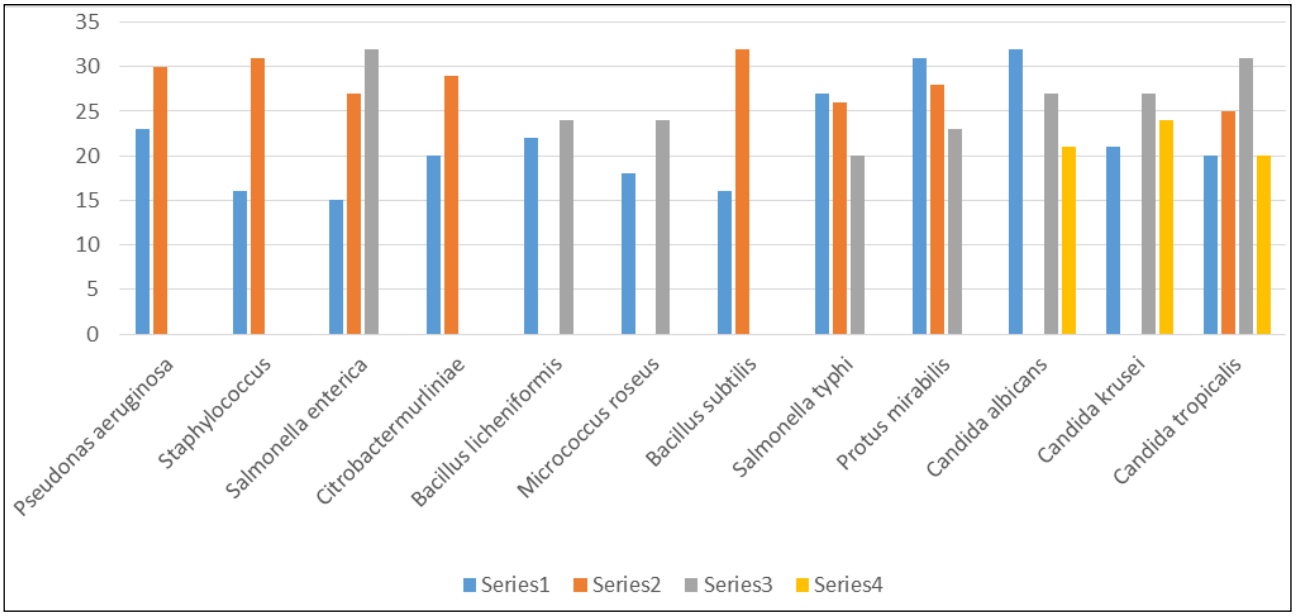

Fig 2: Zone of inhibition and antibacterial activities of *Beta vulgaris* leaves against standard drugs

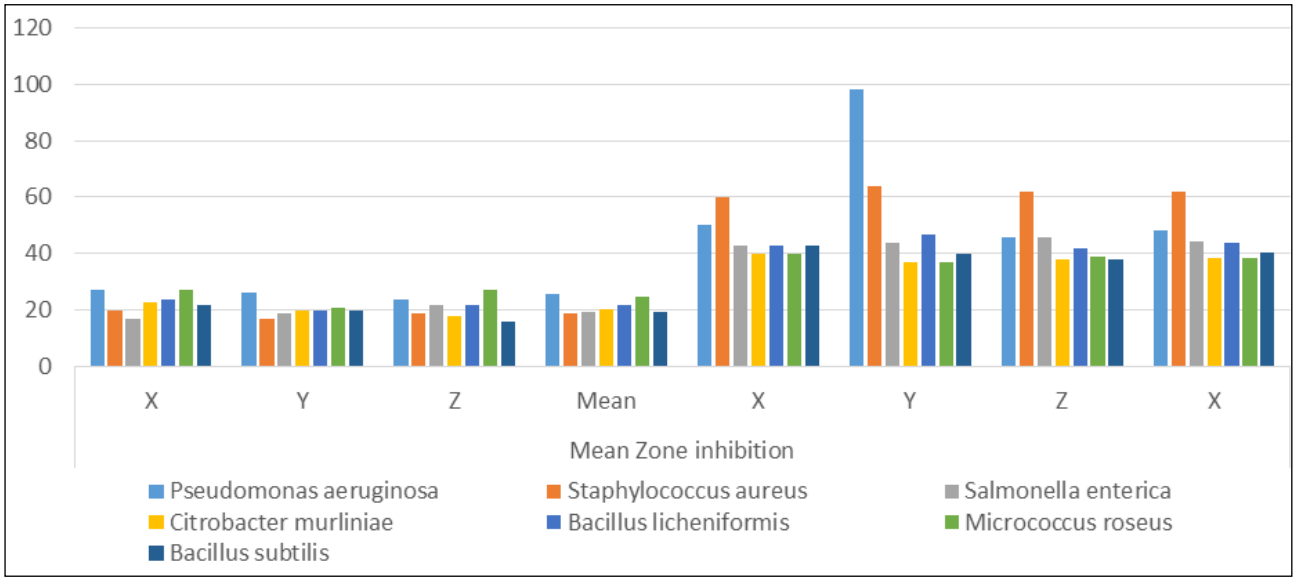

Fig 3: Zone inhibition and antimicrobial activities of *Phoenix dactylifera*

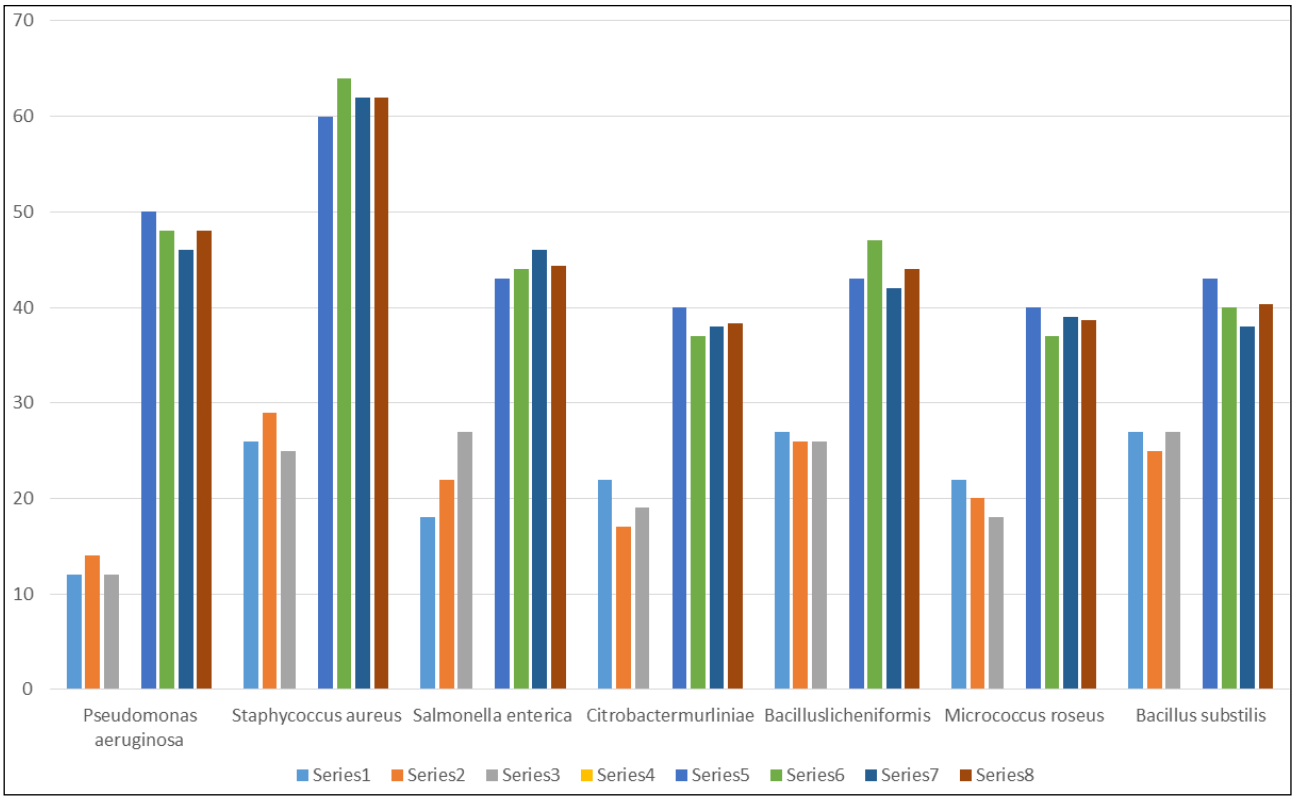

Fig 4: Preliminary antimicrobial of 1000mg/L susceptibility screening of the methanol extracts of *Zingiber zerumbet* (Bitter Ginger)

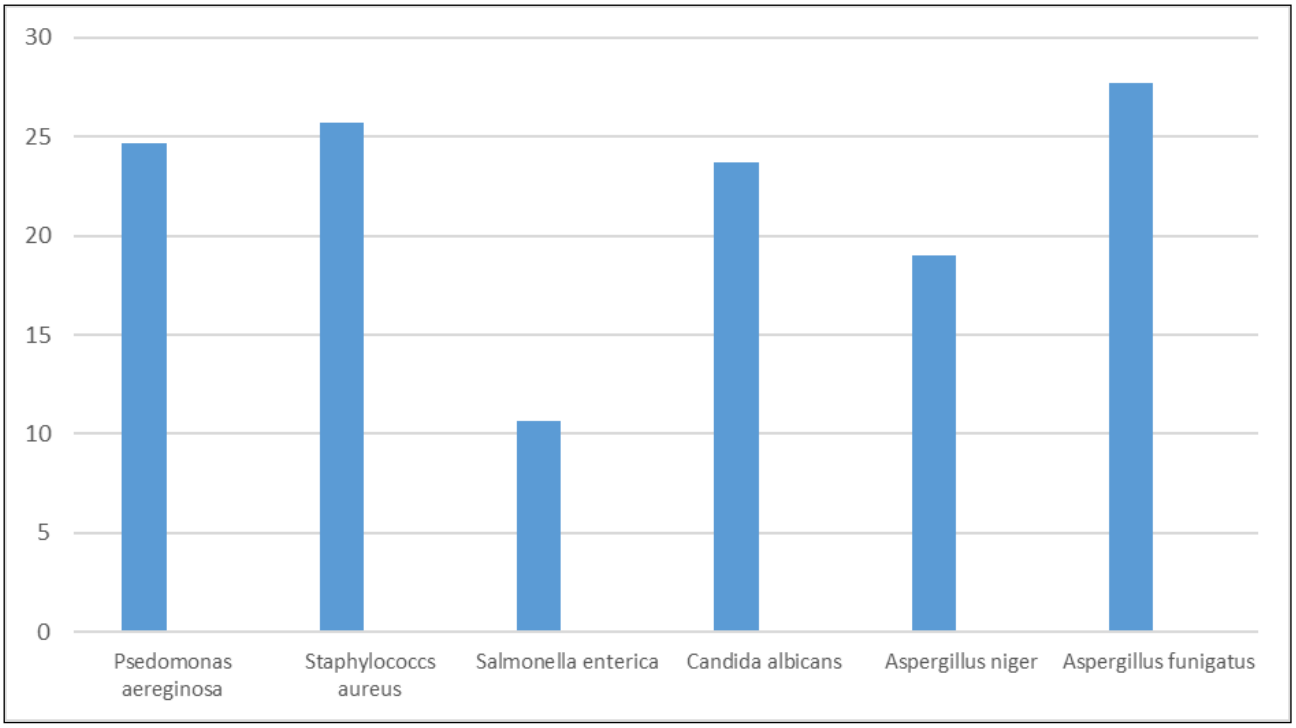

Fig 5: Zone of inhibition of antimicrobial analysis of the ethyl acetate extract of *Curcuma longa*

Antimicrobial Test

Results from the antimicrobial test for the hexanolic extracts of the four samples investigated in the study are summarised in tables 2-5. The results showed that the hexanolic extracts of the four medicinal plants were active all the test organisms. The zones of inhibition for the *Beta vulgaris* ranged from 15-32mm with *Candida albicans* the highest zone of inhibition and *salmonella enterica* the least. *Phoenix dactyliferal* recorded a mean zone of inhibition between18.7-25.7mm with *Staphylococcus aureus* the lowest zone of inhibition and *pseudomonas aeruginosa* the highest. The activities of *Zingiber zerumbet* against the pathogens also recorded high zones of inhibition as *Staphylococcus aureus* has the highest zone of inhibition (26.6mm) and *pseudomonas aeruginosa* the least (12.7mm). The investigation on *Curcuma longa* against the clinical pathogens was also an interesting report as all organisms were active to the extract with *Aspergillus fumigatus* the highest zone of inhibition and *Salmonella enterica* the lowest (27.67 and 10.67mm) respectively. The fact the

extracts could inhibit the growth of the test organisms could be attributed to the presence of some natural products. Some bioactive compounds such as alkaloids, flavonoids, saponins have been reported to show antimicrobial activity against some clinical Gram positive and Gram-negative pathogens (Nna *et al*., 2018, 2021) [6]. Sofowora (2008) also reported that *Pseudomonas aeruginosa* and *Staphylococcus aureus* were active against *Euphorbia hirta Linn* extract. The fact that the four plants under investigation show high activities on the pathogens investigated imply that they could be used as a precursor in managing or treating diseases caused by the pathogens. *S. aureus* has been reported to carry necrosis and toxic shock syndrome (TSS) Novick *et al*., (1979). The plants can also be used against urinary, respiratory tracts infections and chest infections which *Ps. aeruginosae* is the causatic agent (Yen and Yang, 1971). Since S. The plants' antibacterial properties also suggest that they could be helpful in treating HIV (Human Immunodeficiency Virus) sufferers. bacteria and Ps. According to reports, aeruginosae have been linked to secondary infections in HIV patients, such as burn and superficial wound infections (Ibrahim *et al*., 2006) [3]

Conclusion

This work has shown that *Curcuma longa, phoenix dactylifera, Beta vulgaris* and *Zimgiber zerumber* contain pharmacologically potential ingredients (phytocompounds). This implies that the plants could be used in treating some ailments. It is obvious that the presence of these compounds could be responsible for activities of the plant extracts under investigation against the clinical pathogens used in the study. This work revealed that all plant extracts under investigation showed high activities against the pathogens examined in the study and inhibited the growth of the pathogens and sensitivity which implies that the plants can cure all diseases caused by the pathogens as such they could serve as useful precursors for antibiotic drugs candidates. This work has shown scientific evidences on the usefulness of the plants investigated in trado-medicines to ascertain its claims by ethno-medical practitioners.

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