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In-Vitro Antimicrobial Efficacy Of Four Herbs Extracted In Methanol Against Pseudomonas Aeruginosa And Bacillus Subtilis.

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Abstract
Background: *The effects, high cost, unavailability of antibiotics, and development of resistance by pathogenic microorganisms against commercial antibiotics, many people have opted to go for herbs. The Kigelia africana (root, bark, and fruit), Carica papaya leaves, Zingiber officinale (ginger) rhizomes, and, Jateorhiza palmitate (Calumba) root from Africa mainly Malawi (usually used as herbal medicines by many people from different parts of the world). This study was undertaken to screen the antibacterial action of Carica papaya leaf extract, Kigelia africana bark, root, and fruit extracts, Jateorhiza palmitate extract, and Zingiber officinale extract against two different pathogenic bacteria and the herb's antibacterial efficacy was compared with three different commercial antibiotics*
Materials and Methods*: Antimicrobial activities of methanol-extracted herbs were examined against two species of pathogenic microorganisms. Pseudomonas aeruginosa and Bacillus Subtilis using paper disks impregnated with antibiotics and herbs were placed in a culture medium and checked for zones of inhibition using a ruler. A total of 81 samples were collected. The randomized block design is analyzed by the two-way analysis of variance (two-way ANOVA) technique and was used to test the efficacy differences between the herbs extracts and commercial antibiotics using Gen stat 18 Edition. The extracts were screened for the presence of alkaloids, saponins, tannins, terpenoids, flavonoids, anthraquinones, and anthocyanin using standard methods described by researchers.*
Results: *The extracts were screened for the presence of alkaloids, saponins, tannins, terpenoids, flavonoids, anthraquinones, and anthocyanin using standard methods described by researchers. The methanol-extracted herb of Carica papaya leaves, the extract has does not affect inhibiting the growth of tested microorganisms with both delusions. Among the plants studied, Jateorhiza palmitate (Calumba) possessed the highest in-vitro bactericidal efficacy on both gram-positive and gram-negative bacteria as it proved to be more inhibitive than any other extract against both B. subtilis and P. aeruginosa having a zone of inhibition of 21.00mm and 25mm, respectively.*
Conclusion: *Phytochemical screening of the samples revealed the presence of some bioactive components like alkaloids, saponins, tannins, anthraquinones, anthocyanin, flavonoids, and terpenes. These phytochemical properties determine the anti-microbial potential of the herbal extracts*
Key Word: *Antibacterial activity, Sausage tree, Calumba, Carica papaya.*

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I. INTRODUCTION

The control of contagious illnesses is extremely endangered with the stable rise in the number of micro-organisms that are a resilient widespread variety of bacterial agents. Resilient infections are central to amplified sickness as well as lengthy hospital admissions. [1] Although the greatest effect, as well as new advanced antimicrobial drugs, are available throughout the biosphere, their use in emerging nations is confined to persons who can afford them. This has led to extensive as well as unrestrained usage of antibiotics [2] by patients who relatively, frequently do not take a complete sequence of treatment because they cannot afford to buy expensive antibiotics. Additional problems in emerging nations are the quality as well as effectiveness of antimicrobial drugs. In other nations, numerous different antimicrobial drugs are manufactured locally. For example, in India, there are above 80 altered brands of ciprofloxacin fluoroquinolone. In the nation of Vietnam, a locally manufactured 500 mg capsule of ciprofloxacin costs 400 dongs (approximately 2 pence)[3] Antibiotic resistance is a form of resistance to a drug hereby some (or, all, less commonly) sub-populations of a microorganism, mainly bacterial species, can live after contact to one or more antibiotics and such pathogens are known as multidrug-resistant (MDR) or more colloquially, superbugs [4]. Antibiotic resistance in bacteria is an extra, life-threatening, developing phenomenon in modern medicine and has appeared to be the single outstanding community health concern of the 21st century. This is particularly so because it relates to pathogenic organisms that cause diseases in human beings [5].

This phenomenon has led to more investigations into alternative ways such as medicinal herbs [6]. Naturally occurring antibiotics may be improved to give semisynthetic derivatives. These frequently differ from their parental compound's antimicrobial action or pharmacological properties. Therapeutic plants are lakes of numerous metabolites as well as provide an infinite source of significant chemicals that have various biological properties as well as represent a rich source from which antimicrobial agents can be obtained [7]. Such naturally occurring medicinal plants having antibacterial properties include Zingiber officinale, Carica papaya, Jateorhiza palmitate, and Kigelia africana. They contain a wide range of chemical compounds commonly referred to as phytochemicals. Ginger (Zingiber officinale), a member of the Zingiberaceae family, is a well-known spice used in daily diets in many Asian countries. In China, ginger has been used to assist with digestion, as well as to help to treat stomach upset, diarrhea, as well as nausea, for more than 2000 years. Ginger is a hot herb and is a useful medicinal agent [8].

Kigelia africana commonly known as the Sausage tree naturally occurs in the tropical regions of South Africa. The fruit of Kigelia africana has been used traditionally as dressings for wounds and ulcers while an infusion of the bark and roots has been used to treat pneumonia [9]. Another important medicinal plant, Carica papaya, fits in the family of Caricaceae as well as has numerous species of Caricaceae as well with various names papaya, pawpaw, pawpaw, papaya, kebaya, wan shou Kuo, chich put, Tinti, as well as fan Kua and so on. Papaya plants part fruit, leaves, seed, roots as well as latex have phytochemical compounds [10]. These plant parts extracts have been utilized as a medicine against a diversity of illnesses. The plant papaya (Carica papaya L.) is commonly found in Indonesia as well as in several parts of Africa. Nearly all parts of the plant can be used by humans for food or medicinal purposes [11].

Calumba which is the common synonym for Jateorhiza palmitate is a dioecious, climbing shrub producing usually perennial woody stems from 2 - 5 meters long from a tuberous rootstock. In some areas, such as Mozambique, the stems can be annual. The tuber is gathered from the wild for local medicinal use [12]. The plant of calumba has a lengthy history of traditional medicinal use and became very popular in Europe, where it was especially valued as a treatment for digestive problems for people with weak stomachs. The plant is cultivated in several countries as a medicinal plant [13].

The hunt for new sources of antibiotics is a worldwide challenge worrying research institutions, academia as well as pharmaceutical companies since numerous infectious agents are becoming resilient to artificial drugs. The condition has been extra complex by the speedy development of multidrug resistance by microorganisms to the antimicrobial agents available. This study was undertaken to screen the antibacterial action of Carica papaya leaf extract, Kigelia africana bark, root, and fruit extracts, Jateorhiza palmitate extract, and Zingiber officinale extract against two different pathogenic bacteria and the herb's antibacterial efficacy will be compared with three different commercial antibiotics.

II. MATERIALS AND METHOD

Study design: For this study to reflect on what was on the ground, a quantitative type of research was used. The quantitative research focused on how effective the herbal concoctions were compared with commercial synthetic antibiotics. The experiment used a completely randomized block design with three replicates.

Study Location: The research was conducted in 104 and 105 Laboratories of Sharda University in Nagar, Uttar Pradesh. in India.

Study Duration: February 2022 to February 2023.

Sample Size: 81 samples

Sample size calculation: To ensure the precision and accuracy of the results, the experiments were done in replicates and a total of 81 samples were used. The efficacy of the herbal extract was done using the average of the replicates compared with the standardized rating of the zone of inhibition in the Kirby-Bauer method of antimicrobial sensitivity testing.

Procedure methodology.

Matured Carica Papaya leaves fresh bark, fruit, and root bark from the Kigelia africana tree, Zingiber officinale rhizome, and Jateorhiza palmate roots were collected from Mwabvi Wild Reserve, Karonga district hospital, Area 25 in Malawi Central Africa. The plant parts were washed under a tap running water and then rinsed with sterile distilled water. They were placed under the shed until dry and then pulverized using sterile laboratory mortar and pestle to obtain a powdered material. The powder was stored in airtight glass containers and protected from sunlight until required for analysis. For sequential extraction, each powdered plant material was weighed (25 g), and the experiment was done in triplicates and placed in extraction thimbles. The thimble plus the contents were placed in the Soxhlet apparatus which was fitted to a 250 ml round-bottomed flask containing 150 ml of methanol. The sample was refluxed for about 72 hours. The solvent methanol was dried under reduced pressure using a rotary evaporator. To obtain a complete dry extract, each plant extract was transferred to a beaker as well as heated at 55°C on a hot plate with non-stop stirring until the crystalline powder was obtained. The dry extract was kept at 4°C in a refrigerator until an assessment of its antimicrobial activities was done. The extraction of each herbal plant material was done in three replicated to get an average percentage yield and a standard deviation. The yield was expressed as a percentage using the following formula: % Yield = Mass of dry extract (g) x 100 Mass of dry powdered sample (g).

Qualitative phytochemical studies:

Jateorhiza palmitate root (Njoka), Carica papaya leaves, Zingiber Officinale tuber, and Kigelia africana (Sausage tree; Mvunguti) fruit, bark, and root were screened for the presence of alkaloids, saponins, tannins, terpenoids, flavonoids, anthraquinones, and anthocyanin's using standard methods described by Harbone (1998), Sofowora (1993) and other researchers. All the phytochemical screening was done at Sharda University 104 Laboratory.

Antibacterial screening

Test microorganisms

All microbial strains used in the study were clinical strains which were kindly provided by Sharda University's bioprocess Laboratory (106) in Nagar Great Noida. The Gram-positive bacteria used was Bacillus. s while the Gram-negative one was Pseudomonas a. were maintained subcultured in nutrient broth at 4o C.

Evaluation of antimicrobial activity

The susceptibility assay was carried out with a 1 mg/ml concentration of each of the extracts. 5mm in diameter punch sterilized paper was immersed in 10 µ extracts for one minute and dried then placed on 20 µl bacteria-streaked surface of NA Petri dishes. The plates were allowed to stand at 4 o C for at least 2 hours before incubation with the test microbial agents. The extracts served as negative controls. Commercial antibiotic solutions of 0.1ml /5mcg of Ciprofloxacin, 0.1 ml / 1mcg of Oxacillin, and 0.1 ml /23.75 mcg of Sulfamethazine served as the positive controls for the bactericidal activity. The aseptically inoculated Petri dishes were incubated in the incubator at 37 ^{0}C and removed after 24 hours. The Petri dishes were checked for zones of inhibition in both the antibiotic and herbal disk and measurements of diameters using a ruler were taken to the closest millimeters on the zones of inhibition and recorded.

Minimum inhibitory concentration (MIC) determination

The aliquots of extract and their dilutions of 50% and 25% were reconstituted by 1:1 dilution using methanol organic solvent for the extracts as well as were put in each disk made on the culture plates previously seeded with the test organisms. The antibacterial potential of the test compound was determined based on the diameter of the zone of inhibition around the disk. To ensure the precision and accuracy of the results, the experiments were done in replicates mean values were obtained. The efficacy of the herbal extract was done using the average of the replicates compared with the standardized rating of the zone of inhibition in the Kirby-Bauer method of antimicrobial sensitivity testing. The assay was performed as outlined above and graphs of the zone of inhibition diameter against the antimicrobial agent. were plotted for each extract

Statistical Analysis:

Data analysis was based on how big the zone of inhibition measured in millimeters of different bacteria from the point of the paper disk which indicated the effectiveness of the medicine. The analysis of variance (ANOVA) to be specifically a two-way ANOVA randomized design was used to test the efficacy differences between the concoction extracts and commercial antibiotics using Gen18 Edition. Data expressed as the mean ± SEM of at least three independent experiments. Two-way ANOVA was used to calculate statistical significance between control and treated groups with a P value < 0.01 considered to be statistically significant.

III. RESULTS

Different parts of a total of four selected medicinal plants [(K. africana (root, bark, and fruit), J. palmitate (root), C. papaya (leaf), and Z. officinale (root)] extracts were tested for the presence of alkaloids, terpenes, tannins, saponins, anthocyanin's, flavonoids and anthraquinones (Table 1).

Table 1: Phytochemical results

SAMPLE	ALKN	TERP	SAPS	FLAV	TANS	ANTHOS	ANTHROS
Z.officinale	-	+	++	+	+	-	-
K. root	+	-	-	++	++	-	-
J. palmate	-	+++	+	++	-	-	-
K. bark	+++	+	-	++	++	-	-
K. fruit	++	+	-	++	++	-	-
C. papaya	-	++	-	+	+++	-	-

Key: +++ large quantities, ++ moderate quantity, + mild and - absent.

Table 2 shows the results of the in vitro antimicrobial efficacy of methanol-extracted herbal concoctions against bacillus. The highest mean value was observed on SuL (30 mm) at 100% zone of diameter, followed by Cipro (25mm), Calumba (21mm) and Papaya did not affect Bacillus. Results indicated that S. root, S. balk, and S. Fruit had no mean difference however, bacillus was sensitive to Calumba (100%) at CIP, OXA, and SuL. Calumba (100%) can be substituted with Cipro (50%). S. balk & S. Fruit (100%) can be substituted by SuL (25%), with mean values of 18.33, 18.67, and 17.33 mm respectively.

Table.2 In vitro-antimicrobial sensitivity testing of B. subtilis to methanol–extracted herbal concoctions compared to commercial antibiotics

Ant-microbial Agent	Zone Dia. (mm) DO 100%	Rating in comparison with			Zone Dia. (mm) DO 50%	Rating in comparison with			Zone Dia. (mm) DO 25%	Rating in comparison with		
		C	O	S		C	O	S		C	O	S
		I	X	U		I	X	U		I	X	U
		P	A	L		P	A	L		P	A	L
Ginger	12	R	I	R	8	R	R	R	6	R	R	R
Calumba	21	S	S	S	13	I	S	I	9	R	R	R
S. root	19	I	S	S	8	R	R	R	6	R	R	R
S. balk	18	I	S	S	14	I	S	I	10	R	I	R
S. Fruit	18	I	S	S	9	R	R	R	7	R	R	R
Papaya	0	R	R	R	0	R	R	R	0	R	R	R
SuL	30	S	S	S	23	S	S	S	17	I	S	S
Cipro	25	S	S	S	21	S	S	S	17	I	S	S
Oxa	0	R	R	R	0	R	R	R	0	R	R	R

Key: R=Resistant, I=Intermediate, S=Sensitive, CIP=Ciprofloxacin, OXA=Oxacillin, SUL=Sulfamethazole.

Key:	**Resistant**	**Intermediate**	**Sensitive**
	(R)	**(I)**	**(S)**
Oxa	≤ 10	≤ 11-12	≥ 13
Cipro	≤ 15	≤ 16-20	≥ 21
SuI	≤ 12	≤ 13-16	≥ 17

Fig. 1 Zone of diameter against antimicrobial agent on *B. subtilis* bacteria.

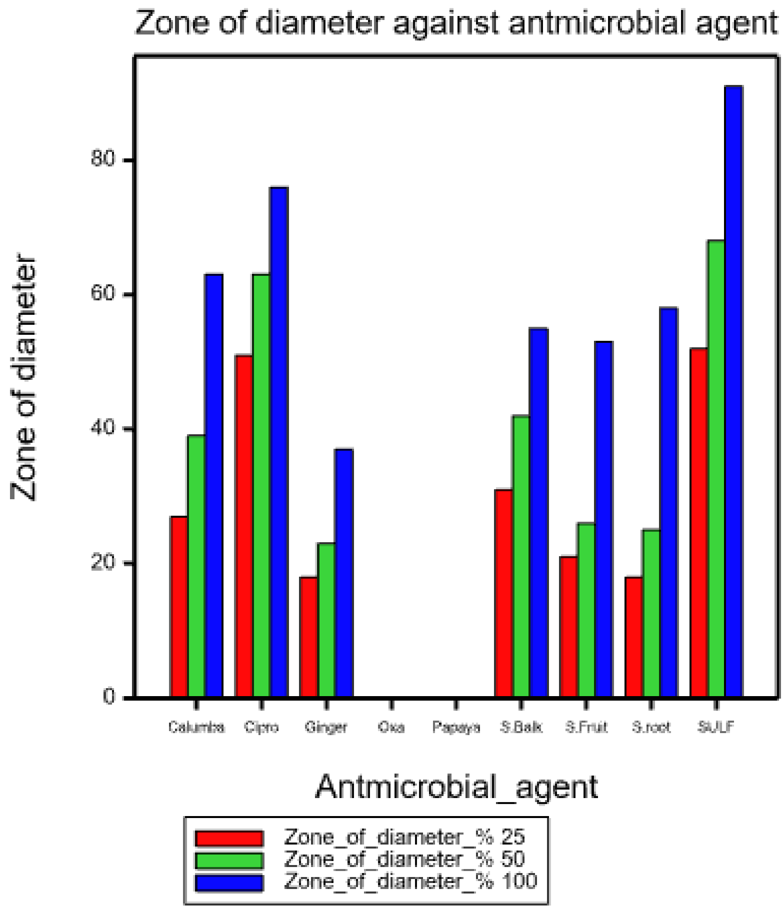


Table 3 shows the results, of the in vitro antimicrobial efficacy of methanol-extracted herbal concoctions against P. *aeruginosa* The highest mean value was observed on Cipro (37 mm) at 100% zone of diameter, followed by Sul (27mm), Calumba (25mm) and Papaya did not affect P. *aeruginosa*. Results indicated that Calumba (25mm) can substitute SUL (27mm). there is no significant difference among S. balk, S. root, S. Fruit, and Ginger (100%), with mean values of 19,20, 21, and 20mm respectively.

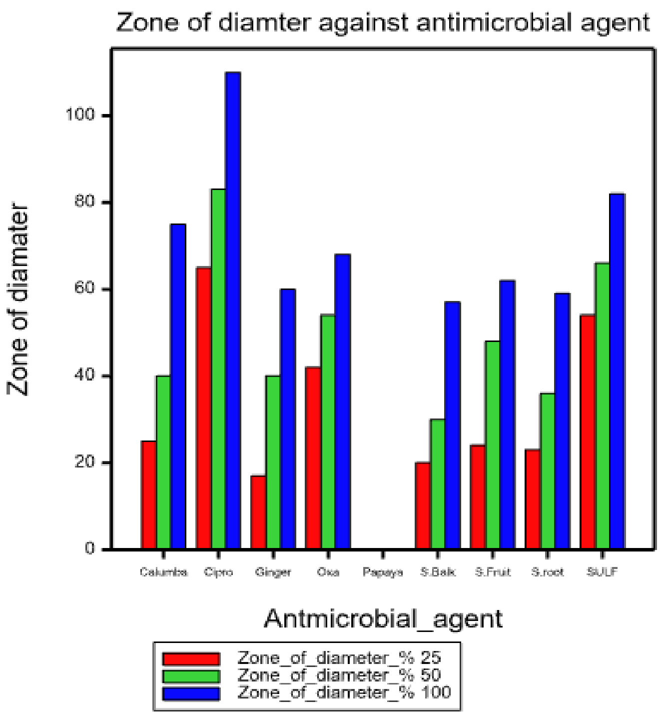
Table 3. In vitro antimicrobial sensitivity test results of P. aeruginosa to methanol-extracted herbal concoctions compared to commercial antibiotics.

Ant-microbial Agent	Zone Dia. (mm) DO 100%	Rating in comparison with			Zone Dia. (mm) DO 50%	Rating in comparison with			Zone Dia. (mm) DO 25%	Rating in comparison with		
		C	**O**	**S**		**C**	**O**	**S**		**C**	**O**	**S**
		I	**X**	**U**		**I**	**X**	**U**		**I**	**X**	**U**
		P	**A**	**L**		**P**	**A**	**L**		**P**	**A**	**L**
Ginger	20	I	S	S	13	I	S	I	6	R	R	R
Calumba	25	S	S	S	13	I	S	I	8	R	R	R
S. root	20	I	S	S	12	R	I	R	8	R	R	R
S. balk	19	I	S	S	10	R	I	R	7	R	R	R
S. Fruit	21	I	S	S	16	I	S	I	8	R	R	R
Papaya	0	R	R	R	0	R	R	R	0	R	R	R
SuL	27	S	S	S	22	S	S	S	18	I	S	S
Cipro	37	S	S	S	28	S	S	S	22	S	S	S
Oxa	23	S	S	S	18	S	S	S	14	R	S	S

Key: R=Resistant, I=Intermediate, S=Sensitive, CIP=Ciprofloxacin, OXA=Oxacillin, SUL=Sulfamethazole.

Key:	**Resistant**	**Intermediate**	**Sensitive**
	(R)	**(I)**	**(S)**
Oxa	≤ 10	≤ 11-12	≥ 13
Cipro	≤ 15	≤ 16-20	≥ 21
SuL	≤ 12	≤ 13-16	≥ 17

Fig. 2. Zone of diameter against antimicrobial agent on *P. aeruginosa*.



IV. DISCUSSION

Photochemistry and Evaluation of the antibacterial potential of C. papaya, Z. officinale, J. palmitate, and K. africana root, bark, and fruit. Phytochemicals and antimicrobial activity were determined in selected medicinal plants namely Z. officinale, C. papaya, J. palmate, and K africana. The tested herbs were found to have tannins, saponins, anthraquinones, anthocyanin, alkaloids, and flavonoids. These results were consistent with results by [1], who tested that the antimicrobial potency of the assay was due to phytochemicals. From the phytochemical analysis results (Table 1), it was observed that Zingiber officinale, and Kigelia (bark, root, and fruit); had a lot more chemicals than the other two herbs. After the laboratory analysis of Kigelia (bark, root ad fruit); it was found that bark contained terpenes, alkaloids, flavonoids, and tannins. K. africana fruit contained alkaloids, flavonoid terpenes, and tannins only whereas K. africana root contained alkaloids, flavonoids, and tannins. These results are in line with the findings of [2] J. palmate contained terpenes, which was consistent with what [3] found in his study. Saponins and flavonoids were also present in J. palmate which is in contrast with what [4] found in his study for he found tannins. Z. officinale contained tannins, terpenes, saponins, and flavonoids which were also reported in [1] study findings. Lastly, C. papaya contained terpenes, flavonoids, and tannins and this result was in line with what [5,6] reported in their study. The bioactive compounds were extracted using methanol in sequence using a Soxhlet extractor for 72 hours for a sample of 25g with 150 ml of each solvent done in triplicates. The existence of bioactive substances has been stated to grant battle to plants to fungi, pests as well as bacteria. [7], Since this clarifies the demonstration of in-vitro antibacterial activity by the plant extracts used in this study meaning that the tested herbs could be used as alternative antimicrobials for some antibiotics [8]. Methanol extract in-extracts of inhibition were compared concerning their antimicrobial activity with some commercially obtained antibiotics (Table 2&3). Zone of inhibition against Bacillus subtilis and Pseudomonas aeruginosa produced by the extracts at 25g were compared to 1ml /5mcg of CIP, 1ml /1mcg of OXA, and 1ml /23.75 of SUL.

The antibacterial activity results showed that most plant extracts were effective in inhibiting the growth of Bacillus. s and Pseudomonas a. with varying inhibitory effects as indicated in (Tables 2&43). But Papaya extract does affection inhibit the growth of bacillus both delusions which were in contrast with what [15,16,17] reported, the methanol plant leaf extracts were more active against gram-positive than gram-negative. This may be so due to differences in bioactive compound extraction methods and environmental factors. According to [30], insufficient amounts of the active element or constituents in the extract to demonstrate antibacterial activity may be the cause of the negative results.

The results are similar to the findings of [24] revealed that fresh green C. papaya extract had no significance on Pseudomonas aeruginosa, with an average mean value of 0 in all zones of diameter. Regardless of the findings, studies show that dry papaya ethanolic leaf extract includes antibacterial components that inhibit the growth of a wide range of Gram-positive and Gram-negative organisms [28]. C. papaya methanol extract showed no zone of inhibition against any bacterial strain. The difference among different studies may be due to the variety of papaya used, or bacteria resistance [25].

Ginger showed intermediate inhibitory compared to OXA, which agrees with the findings of [27], due to the presence of terpene. Among the plants studied, methanol-extracted calumba possessed the highest in-vitro bactericidal efficacy as it proved to be more inhibitive than any other extract against both B. subtilis and P. aeruginosa having a zone of inhibition of 21.00mm and 25mm, respectively. The ginger extract showed antibacterial activity (6 – 10), which is consistent with the findings of [29], who stated that methanol extracts demonstrated significant antibacterial activity and formed a zone of inhibition ranging from 10 to 28 mm. Ginger kills Multi-Drug Resistant P. aeruginosa clinical isolates by altering cellular physiology and preventing biofilm formation [22].

These results collaborate with [8] who also studied the antibacterial activity of Calumba extract. The methanol crude extracts of Calumba had substantial in-vitro effects on both B. subtilis and P. aeruginosa unlike when it was diluted to 50% (1:1 from 100%) and 25% (1:1 from 50%). The in-vitro antimicrobial activity of methanol Calumba extract on both strains of bacteria is clear evidence that bioactive substances work as suicidal agents. This was proved when the zone of inhibition diameter of Calumba was compared to the standard rating of diameters of CIP, OXA, and SUL [9]. From Tables 2 &3 it can be observed that Calumba was as good as all three commercial antibiotics in terms of strength since its sensitivity was as good as that of the three synthetic antibiotics on both bacteria implying that Calumba can be an alternative for the three antibiotics.

The high values from Calumba methanol extract for B. subtilis and P. aeruginosa were a good indication of high in-vitro efficacy against these bacteria. This outcome was remarkable, considering that upper respiratory tract infections caused by B. subtilis and enteric infections caused by P. aeruginosa are on the rise and also becoming resistant to first-line antibiotics especially commonly used antibiotics as observed from results on B. subtilis with Sulfamethoxazole as treatment in developing countries as observed by [10,11]. Therefore, Calumba can be an alternative for both narrow and broad-spectrum antibiotics [12,13,26] revealed the higher the concentration of Calumba extract the higher the bacterial growth inhibition, and the results showed Calumba under the highest bacteria growth inhibition was achieved. This assures that calumba can provide a valuable antimicrobial agent for responding to infectious diseases caused by Bacillus subtilis as suggested by [23].

From the results of the study, it can be demonstrated that crude methanol extract of K. bark would inhibit the effect of B. subtilis in vitro giving credence to K. bark to be used as a narrow spectrum antimicrobial to B. subtilis infections with a zone of inhibition of 19mm on P. aeruginosa and 18mm on B. subtilis. These findings were in line with what [14] found in their study. When its efficacy was compared with commercial antibiotics, K. bark was as sensitive in vitro as OXA and SUL against B. subtilis as well as P. aeruginosa and as an intermediate, if compared with CIP against B. subtilis and P. aeruginosa. africana bark could be used as a broad-spectrum antimicrobial against P. aeruginosa and as a narrow-spectrum antibiotic against B. subtilis.

A methanol-extracted concoction of K. africana fruit showed in vitro bactericidal activity. B. subtilis and P. aeruginosa This was not in line with the results of the study done by [14]. In which, their study K. african fruit was inhibiting B. subtilis only. The findings of S. Fruit's antibacterial activities are similar to those of [21], who discovered that methanol extracts of S. Fruit exhibited antibacterial activity on both gram-negative and gram-positive bacteria. The bactericidal activity of the S. Fruit extract ranged from 0 to 11mm in diameter [20].

When its efficacy was compared with commercial antibiotics, K. africana fruit was as sensitive in vitro as OXA and SUL against B. subtilis as well as P. aeruginosa and as an intermediate if compared with CIP against B. subtilis and P. aeruginosa. Therefore, K. african fruit could be used as an alternative for OXA, CIP, and SUL depending on the severity of the infection.

A methanol-extracted concoction of K. root was active in vitro against both B. subtilis and P. aeruginosa which was in contrast to what [18,19] found in their study, where the root was only active against B. subtilis This may be so due to differences in bioactive compound extraction methods and environmental factors. If compared with commercial antibiotics, the K. root was as sensitive in vitro as OXA and SUL against the strains of bacteria. As intermediate when compared with CIP.As such K. root can be used as an alternative for CIP, OXA, and SUL.

V. CONCLUSION

The study has demonstrated the in vitro activity of K. africana, C. papaya, J. palmate, and Z. officinale against both Gram-negative and Gram-positive bacteria. This indicates that the plants would be a potential source for the production of drugs with a broad spectrum of activity as in the case of methanol-extracted calumba concoction results and a narrow spectrum as in the n case of methanol-extracted K. africana fruit concoction. The results of the study confirmed the traditional use of the tested plants and suggest that plant extracts possessed compounds with antibacterial properties that can be used as antibacterial agents as alternative drugs for the treatment of gastroenteritis and upper respiratory tract minor ailments.

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