

RESEARCH ARTICLE

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Comparative Study of the Antibiotic Potency of Natural (*Cyanthillium cinereum* and *Moringa oleifera*) and Synthetic (Ampicillin and Erythromycin) Antibiotics

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Abstract

The aim of this study was to compare the antibiotic potency of *Cyanthillium cinereum* and *Moringa oleifera* (natural antibiotics) against Ampicillin and Erythromycin (synthetic antibiotics) using zone of inhibition and both the natural and synthetic antibiotic was treated against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The extracts from the natural agents were obtained by the use of methanol (polar) and hexane (non-polar). The bacteria were treated with six different conc. (100%, 75%, 50%, 25%, 10% and 5%) of the extracts, where the largest zone of inhibition was observed with *C. cinereum* extract at 5% concentration against *S. aureus* with mean and standard deviation of 22.1 ± 2.1 . *S. aureus* followed by *P. aeruginosa* was most susceptible towards the treatments. The correlation (Pearson) indicated that the conc. of the extract was indirectly proportional to the zone of inhibition. The phytochemical analysis revealed that *C. cinereum* contained alkaloids, catecholic tannins, saponins, flavones, volatile oils phenols and steroids and *M. oleifera* only contained Alkaloid, tannins and steroids.

Keywords: Synthetic, antibiotic potency, natural drugs, Synthetic antibiotics, zone of inhibition

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(Received: October 14, 2019; accepted: January 10, 2020)

Citation: Tresonne Headley, Ruth Daniel, Elford Liverpool and Abdullah Adil Ansari, Comparative Study of the Antibiotic Potency of Natural (*Cyanthillium cinereum* and *Moringa oleifera*) and Synthetic (Ampicillin and Erythromycin) Antibiotics, *J. Pure Appl. Microbiol.*, 2020; **14(1)**:287-300. <https://doi.org/10.22207/JPAM.14.1.30>

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INTRODUCTION

Bacteria are single-cell organisms that have been in existence for millions of years (Encyclopedia Britannica, 2017). Since their existence they have adapted the ability to inhabit virtually everywhere; from the harshest of climates to the most suitable of environments (including the human body); thus resulting in the formation of several bacterial species (Rogers and Kadner, 2017). Bacteria are classed based on their difference shapes, sizes and even method of locomotion (Vidyasagar, 2015). Bacteria could be either spiral, spherical or rod-shaped, and could be transmitted through several sources; some are airborne while others are found either in water or soil (Cox, 2016).

The human body poses a suitable environment that is rich in nutrients, thus it attracts both good and bad bacteria. Not all bacteria are bad; there are good bacteria or otherwise called beneficial bacteria that lurk in, on the surface of the human body and even aids in making some of the desiring foods for humans, for example, *Lactobacillus johnsonii* is a beneficial bacteria that is found in the gut and makes digestion of milk smoother (Devault, 2017).

Infections are responsible for several diseases that compromises the health of human beings; especially humans with weak immune systems. Infectious diseases such as (cold, flu and AIDS), (malaria), (urinary tract infection, meningitis and tuberculosis) and (ringworm and athlete's foot) can be caused by viruses, parasites, bacteria and fungi respectively; however, bacterial disease infections are mostly common (Explorable, 2010). With the exception to infections caused by viral diseases (such as AIDS, flu and bronchitis); infections can be averted, managed and treated through several antimicrobial compounds, which is collectively referred to as antibiotics (Explorable, 2010). Most of the antibiotics are referred to "microbicsins", substances (medicines) which cause one microorganism to manage or kill another and in the same process causes a minute or no harm to normal body cells (VIHA, 2014). The word antibiotic is more so related to bacteria (i.e. substances that manage/kill bacteria). With that said, there are several mechanisms by which antibiotics can complete such tasks; there are the

inhibition of the cell wall synthesis, inhibition of protein synthesis, inhibition of folate synthesis or binding DNA gyrase (VIHA, 2014).

As Antibiotics are classified into two board groups; bactericidal and bacteriostatic antibiotics, first basically impedes with the bacterium's cell wall formation or its cell contents, thus killing the bacterium and the other inhibits protein production, DNA replication and several other cellular metabolism, thus the bacteria loses the ability to multiple¹. Penicillin, daptomycin, metronidazole, nitrofurantoin are all bactericidal antibiotics, whereas, the bacteriostatic antibiotics are tetracyclines, spectinomycin, chloramphenicol and trimethoprim. However, these are all substances that have been synthesized over the years and must be taken at the right lengths of time or used for the appropriate bacterial infection to avoid the bacteria from becoming resistant to the antibiotics, but the indiscriminate use and easy accessibility of such antibiotics to date, is causing an significant increase to the problem which is the rapid development and multiplication of antibiotic resistant bacteria³. There are several mechanisms adapted by bacteria which allows them to withstand the effects of the synthetic antibiotics; mechanisms such as the inactivation of the antibiotic, alteration of the target sites and membranes, and efflux pumps². The aim of the work will be to compare the antibiotic potency of *Cyanthillium cinereum* and *Moringa oleifera* (natural antibiotics) against Ampicillin and Erythromycin (synthetic antibiotics) using zone of inhibition. The Objectives of this will be to investigate the potency of *Cyanthillium cinereum* as a natural antibiotic agent against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, to see the potency of *Moringa oleifera* as a natural antibiotic agent against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and finally to compare the antibiotic potency of the natural agents (*Cyanthillium cinereum* and *Moringa oleifera*) against that of the selected synthetic antibiotics (Ampicillin and Erythromycin).

As we have a decrease in the antibiotic properties of synthetic drugs and Guyana is filled with a rich diversity of fauna one has to tap into this filed for alternate forms of cure for diseases.

This will enable locals to use more of natural crops or use plants with medicinal properties for consumption on a regular basis.

MATERIALS AND METHODS

This project (comparative study of the antimicrobial effectiveness of natural and synthetic antibiotics) was carried out at the University of Guyana Turkeyen Campus, in the Department Biology laboratory.

Collection of Plant specimens

The Whole *Cyanthillium cinereum* (Inflammation bush) and *Moringa oleifera* (Saijan plant); with the exception of the roots and fruits, were hand-picked during the month of June, 2017. The plants were collected from various Regions around Guyana. The *Cyanthillium cinereum* (Inflammation bush) was collect from Region 3 (La Grange West Bank Demerara) and Region 4 (Betervverwagting East Coast Demerara/Demerara-Mahaica). The *Moringa oleifera* (Saijan plant) was collected from Region 4 (Turkeyen East Coast Demerara/Demerara-Mahaica) and Region 3 (Tuschen Essequibo-West Demerara).

Ms. Kaslyn Collins (Scientific Officer -Botanist) of the Center for the Study of Biological Diversity (CSBD) had Identified and confirmed the plant *Cyanthillium cinereum* and *Moringa oleifera* to carry out the work.

Preparation of Extracts

Healthy plants were collected, they were cleaned and washed thoroughly to ensure the removal, of pests or eggs. The plants were then placed into the oven at 45-50°C for 1-2 weeks to be dried. The dried plants were powdered by a mill, for the preparation of extracts. Both polar (methanol) and non-polar (hexane) solvents were incorporated in the extract process. Approximately, 1320g and 110g of the powdered *Cyanthillium cinereum* were soaked in 1500mL and 1250mL of 95% methanol and hexane respectively and 46.82g of the powdered *Moringa oleifera* was soaked in 750mL of 95% methanol and hexane. After the 24 hours soaking period, the extracts were shaken vigorously and then strained. They were placed into glass containers and kept at room temperature.

The extracts were placed into the rotary evaporator to remove the solvents used. The rotary evaporator reduced the extracts down to a

semi solid state. By reducing the extract to a semi solid state, most if not all of the extracting solvents used were allowed to evaporate from the samples and a very concentrated product remained inclusive of the various phytoconstituents which is known as the crude extract. The hexane (alkane) extracts evaporated at a much faster rate than the methanol (alcohol) extracts since it has a lower boiling point (69°C) than methanol which has a slight higher boiling point of 78°C. After the crudes were obtained, they were collected and stored in tightly capped glass vials.

Preparation of plates using Mueller Hinton Agar Procedure

27g of Muller-Hinton agar was added into a conical flask to which there after 700mL of water was added. The mixture was swirled gently and then placed onto the hot plate for approximately ½ hour, which allowed the agar to dissolve further. At timely intervals, the flask was swirled and the pressure build-up within the flask was lowered by removing the cap. As bubbles emerge from the bottom of the flask, the solution was removed from the hot plate and placed into an autoclave at 121°C for fifteen minutes at 12psi to be sterilized. After the solution was autoclaved, it was cooled under running tap water to around 55°C before being poured into the petri dishes. To reduce the risk of contamination, the agar was poured inside a biosafety laminar air-flow cabinet. After pouring, the plates were left to sit at room temperature for under an hour to solidify. After solidification, they were UV sterilized a second time for 20 minutes so as to ensure that there were no possible contamination. The plates were then inverted and placed into the refrigerator were they stood overnight. After such, the plates were placed inside an incubator in an effort to remove any excess moisture that may have accumulated from the fridge, then they were labeled and there after the respective organism was streaked on to the plates. Bacteria was obtained from the Faculty of Health Sciences at the University of Guyana based on their pharmacological and clinical importance and the other was obtained from the Department of Natural Sciences at the University of Guyana. NB. Microbes were maintained at 15°C on nutrient plates. Viz: *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*).

Preparation of McFarland standard (Turbidity)

In an effort to ensure that the appropriate among of microbial organisms were allocated to the agar Conical flask

Approximately 85ml of 1% sulphuric acid was poured into a conical flask. With the use of a volumetric pipette 0.5ml of 1.1175% barium chloride was then added to the conical flask. Soon after 1% sulphuric acid was poured into the conical flask to bring the volume up to 100ml and it was stirred until there were no clumps present. 10ml of the barium sulphate precipitate was poured into two test tubes and they were capped using paper caps. The test tubes were securely wrapped in aluminum foil and kept at room temperature.

Disc Diffusion Test

Procedure

Under good aseptic conditions, the prepared standard the test tubes containing 10ml sterilized water were placed into the cabinet. An inoculating loop was flamed red hot and allowed to cool. It was used to gently remove a small amount of bacteria culture from the sub-cultures (either *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* or *Staphylococcus aureus*) and was placed into one of the test-tubes containing 10mL sterilized water. NB. The tube was flamed after the stopper was removed and before it was recapped.

The test tube was shaken gently in a side to side manner, thus allowing the bacteria to better diffuse throughout the solution. It was then compared with the McFarland turbidity solution. As long as the content of the prepared tube appeared similar to that of the McFarland solution the appropriate label was placed onto the tube. This above steps were done for the two other bacteria species. On removal of the cotton stopper, the tube was flamed and the sterile cotton applicator was dipped into the test tube containing the bacterial culture of known and then swabbed uniformly onto the surface of the agar and was allowed to dry for 5 minutes.

5mm discs were punched from Whatman no 1 filter paper using a paper puncher after which they were sterilized in a petri plate under ultra violet light provided by the laminar air-flow cabinet for 20 minutes. The discs were then placed into petri dishes containing the 100% concentrations of methanol and hexane extracts of the selected plants. In a similar manner negative controls were

prepared; they were allowed to soak in the pure extraction solvents (methanol and hexane). The positive control for the bacteria being the both the 500mg Ampicillin and 500mg Erythromycin in 20ml sterilized water, the filter paper disks were soaked in both the Ampicillin and Erythromycin solutions.

The soaked disks along with the negative control and the positive controls, the ampicillin and erythromycin antibiotic discs were arranged on the agar plate with equal spacing between each disc via the use of sterile tweezers. This was repeated for each bacteria with 100%, 75%, 50%, 25%, 10% and 5% concentrations of methanol and hexane extract of *Cyanthillium cinereum* and *Moringa oleifera*. The plates were kept for incubation at 37°C for 24 hrs. At the end of incubation period, inhibition zones formed around the disk were measured with transparent ruler in millimeter. NB. These studies were done in triplicates.

Phytochemical analysis

Phytochemical analysis were carried out on all extracts to note its content within each plant.

Alkaloids

2 ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids (Wadood, 2013).

Tannins

To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A green colour indicates for catecholic tannins (Wadood, 2013).

Saponins

0.5 ml of extract was measured in a test tube and was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of Saponins (Wadood, 2013).

Flavonoids

4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and magnesium ribbon was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and an orange colour for flavones (Wadood, 2013).

Volatile oils

2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present (Wadood, 2013).

Phenols

1ml of extracts was treated with 3-4 drops of ferric chloride solution. Formation of bluish-black colour indicates the presence of phenols (Wadood, 2013).

Steroids

1ml of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly. A green bluish colour indicated positive for steroids (Wadood, 2013).

RESULTS AND DISCUSSION

It can be seen that the *C. cinereum* extract of the methanol showed positive for all the phytochemicals (alkaloids, catecholic tannins, saponins, flavones, volatile oils phenols and steroids) as compared to the other extracts (Table 1).

The methanol and hexane extracts from *Cyanthillium cinereum* (Inflammation bush) and

Moringa oleifera (Saijan plant) were tested against selected bacteria to observe their inhibitory effect on the growth of the bacteria colonies. The mean and standard deviation for each extract was calculated in addition to ANOVA two-factor without replication analysis was conducted on each extract using the Microsoft Excel 2013.

The largest zone was obtained with the hexane extract with a mean of 22.1mm at 5% concentration and the smallest zone was 6.9mm at 75%. However, the largest zone for methanol extract was 14.8mm at 5% and the smallest was 10.6mm at 25% (Table 2). The P-value obtained from the ANOVA two-factor without replication analysis was 0.47, which means that the extracts of the *C. cinereum* were not statistically significant in zones observed for the *S. aureus* (Table 3).

The largest zone was obtained with the methanol extract with a mean of 14.9mm at 5% concentration and the smallest zone for methanol was 1.3mm at 25%. The hexane's largest zone was

Table 1. Results obtained during phytochemical analysis of the various extracts

Phytochemical test	Sample 1: <i>C. cinereum</i>	Sample 2: <i>M. oleifera</i>	Sample 3: <i>C. cinereum</i>	Sample 4: <i>M. oleifera</i>
	+ Methanol	+ Methanol	+ Hexane	+ Hexane
Alkaloids	Present	Present	Absent	Absent
Tannins (Catecholic-green)	Present	Absent	Present	Present
Saponins	Present	Absent	Present	Absent
Flavones (orange)	Present	Absent	Present	Absent
Volatile oils	Present	Absent	Absent	Absent
Phenols	Present	Absent	Absent	Absent
Steroids	Present	Present	Present	Present

14.4mm at 5%. The smallest for the hexane was 3.8mm at 75% (Table 4). The P-value obtained from the ANOVA two-factor without replication analysis was 0.05, which means that the extracts of the *M. oleifera* were statistically significant in zones observed for the *S. aureus* (Table 5).

The largest zone was observed with the hexane extract with a mean of 14.6mm at 10% concentration where the smallest being 0.0mm at 50, 75 and 100%. However, the largest zone for the methanol was 11.3mm at 5% conc. and the smallest being 3.6mm at 75% conc. (Table 6). The P-value obtained from the ANOVA two-factor

Table 2. Mean and Standard deviation for methanol and hexane extracts for *C. cinereum* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *S. aureus*

Conc.	% zone of inhibition (mm)	
	Methanol	Hexane
5	14.8±1.8	22.1±2.1
10	13.3±1.2	13.0±5.2
25	10.6±1.6	16.0±3.4
50	12.3±2.0	11.4±4.4
75	14.2±0.9	6.9±5.1
100	13.1±3.8	9.8±3.3

without replication analysis was 0.14, which means that the extracts of the *C. cinereum* were not statistically significant in zones observed for the *P. aeruginosa* (Table 7).

The largest zone was obtained with the methanol extract with a mean of 13.3mm at 5% conc., where the smallest zone for methanol was 1.0mm at 100% conc. The hexane's extract had

Table 3. Results obtained from the ANOVA analysis for methanol and hexane extracts for *C. cinereum* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *S. aureus*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	78.8975	5	15.7795	1.070559	0.471091	5.050329
Within group	0.0675	1	0.0675	0.00458	0.948669	6.607891
Error	73.6975	5	14.7395			
Total	152.6625	11				

Table 4. Mean and Standard deviation for methanol and hexane extracts for *M. oleifera* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *S. aureus*

Concen.		% zone of inhibition (mm)
Concen.	Methanol	Hexane
	5	14.9±0.9
10	13.2±2.0	13.2±1.9
25	1.3±2.4	12.3±1.4
50	1.9±2.8	9.4±4.6
75	1.9±2.8	3.8±4.1
100	4.5±2.9	5.6±5.0

a zone of 13.1mm at 5%. (Table 8). The P-value obtained from the ANOVA two-factor without replication analysis was 0.03, which means that the extracts of the *M. oleifera* were statistically significant in zones observed for the *P. aeruginosa* (Table 9).

The largest zone was observed with the hexane extract with a mean of 10.6mm at 50% concentration with 9.3mm at 5% and the smallest for the extract was 0.0mm at 100% conc. the methanol extract had zone of 9.8% at 5% conc. and 5.3% at the 100% conc. (Table 10). The P-value obtained from the ANOVA two-factor without replication analysis was 0.36, which means that the extracts of the *C. cinereum* were not statistically significant in zones observed for the *K. pneumoniae* (Table 11).

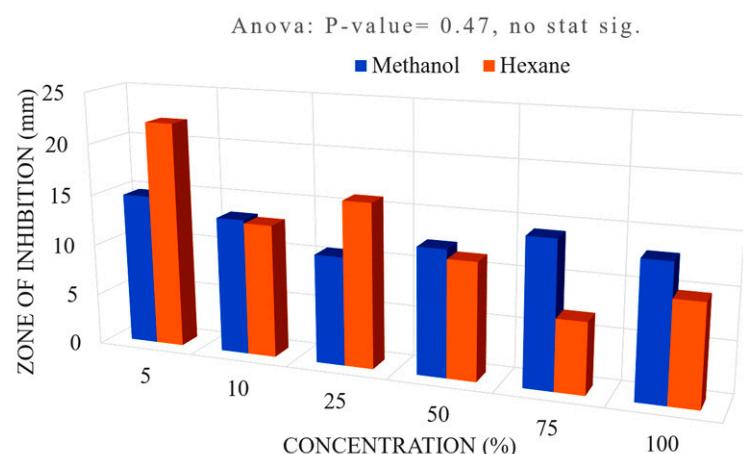


Fig. 1. Difference in zone of inhibition at different conc. for methanol and hexane extract of *C. cinereum*

Table 5. Results obtained from the ANOVA analysis for methanol and hexane extracts for *M. oleifera* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *S. aureus*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	118.8942	5	23.77883	4.815966	0.054747	5.050329
Within group	29.7675	1	29.7675	6.028861	0.057555	6.607891
Error	24.6875	5	4.9375			
Total	173.3492	11				

Table 6. Mean and Standard deviation for methanol and hexane extracts for *C. cinereum* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *P. aeruginosa*

Concen.	% zone of inhibition (mm)	
	Methanol	Hexane
5	11.3±3.1	12.9±3.2
10	10.6±2.4	14.6±3.3
25	11±2.5	12.9±3.9
50	10.4±1.2	0.0±0.0
75	3.6±3.8	0.0±0.0
100	7.8±4.1	0.0±0.0

The largest zone was observed with the hexane extract with a mean of 12.8mm at 5% concentration and the smallest for that extract was 0.0mm at 100% conc. The largest and smallest zone for methanol were 8.4mm and 2.2mm respectively for 5% and 100% conc. (Table 12). The P-value obtained from the ANOVA two-factor without replication analysis was 0.05, which means that the extracts of the *M. oleifera* were

statistically significant in zones observed for the *K. pneumoniae* (Table 13).

The analysis in Table 14 which shows the correlation (Pearson) between the solvents and the concentration was analyze using the Microsoft Excel 2013 and Statistix 10.0 (2013). Where the hexane extracts for both plants showed significant correlation with the zone of inhibition against all three bacteria. However, the methanol extracts showed significant correlation with the zone of inhibition against only *P. aeruginosa* for both plants and *K. pneumoniae* for *M. oleifera*.

NB. The values for the zone of inhibition in the three Figures is the mean values of the four treatments.

Fig. 7 indicates that the Ampicillin was more significantly effectiveness against *S. aureus* then the rest of treatments, followed by Erythromycin, then *C. cinereum* and the least effective being *M. oleifera*.

Fig. 8 indicates that Ampicillin and Erythromycin showed a more significant effectiveness against *P. aeruginosa* with inhibitory

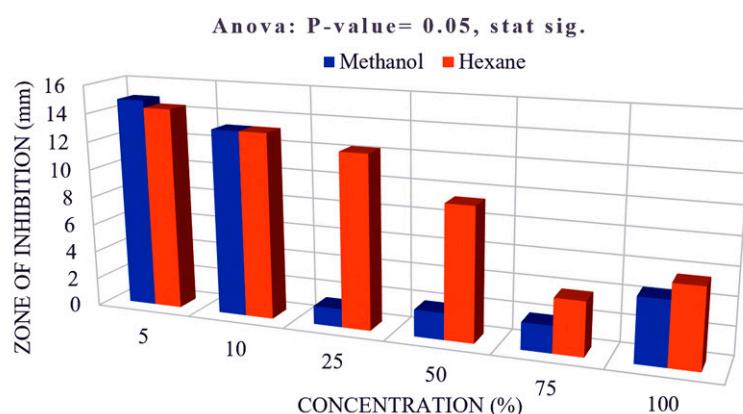
**Fig. 2.** Difference in zone of inhibition at different conc. for methanol and hexane extract of *M. oleifera*

Table 7. Results obtained from the ANOVA analysis for methanol and hexane extracts for *C. cinereum* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *P. aeruginosa*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	233.2575	5	46.6515	2.743426	0.146116	5.050329
Within groups	17.04083	1	17.04083	1.002117	0.362753	6.607891
Error	85.02417	5	17.00483			
Total	335.3225	11				

ANOVA: P-value= 0.14, no stat sig.

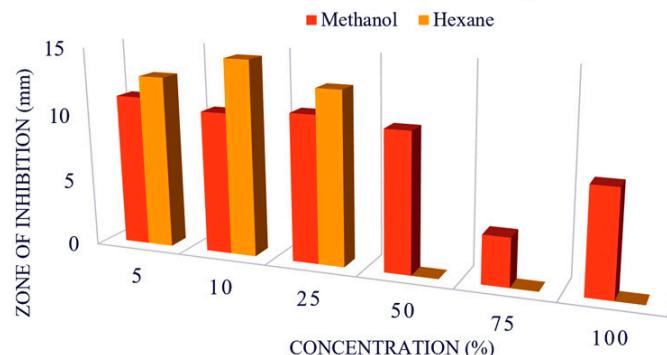


Fig. 3. Difference in zone of inhibition at different conc. for methanol and hexane extract of *C. cinereum*

ANOVA: P-value= 0.03, stat sig.

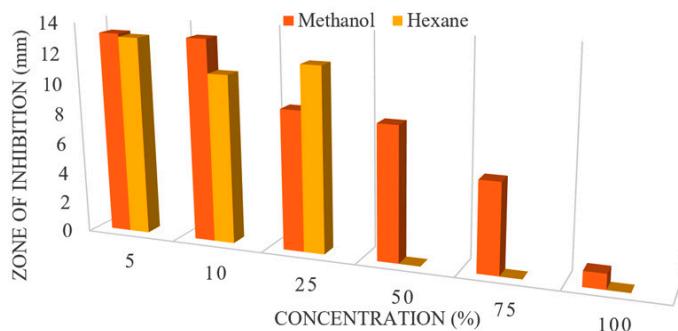


Fig. 4. Difference in zone of inhibition at different conc. for methanol and hexane extract of *M. oleifera*

ANOVA: P-value= 0.36, no stat sig.

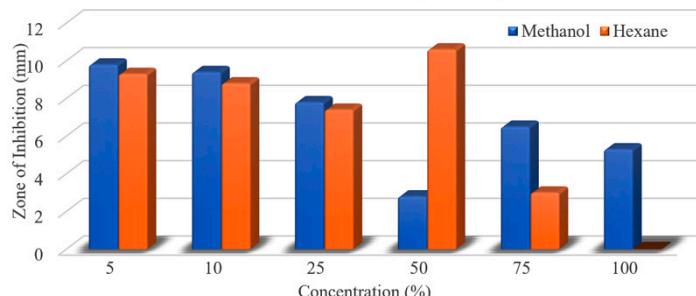
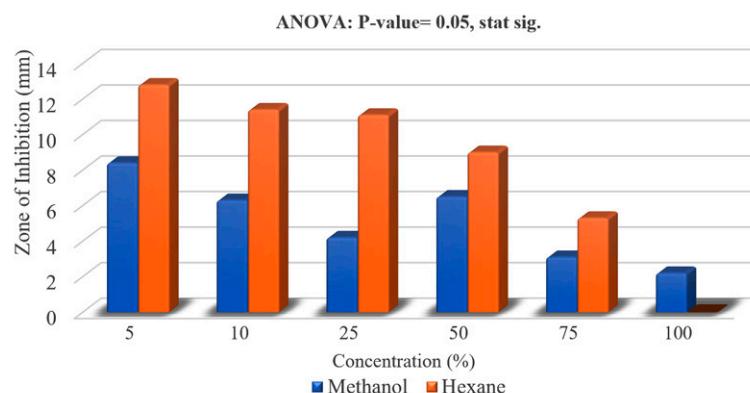
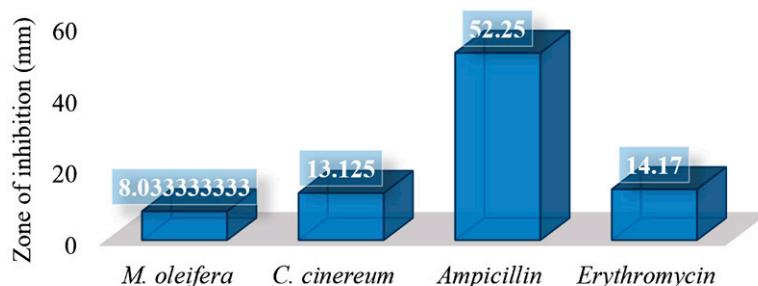


Fig. 5. Difference in zone of inhibition at different conc. for methanol and hexane extract of *C. cinereum*

Table 8. Mean and Standard deviation for methanol and hexane extracts for *M. oleifera* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *P. aeruginosa*

Concen.	% zone of inhibition (mm)	
	Methanol	Hexane
5	13.3±3.2	13.1±2.8
10	13.3±3.5	11.1±3.8
25	9.2±3.4	12.1±2.1
50	8.8±3.4	0.0±0.0
75	5.9±5.2	0.0±0.0
100	1.0±2.3	0.0±0.0

**Fig. 6.** Difference in zone of inhibition at different conc. for methanol and hexane extract of *M. oleifera***Fig. 7.** Difference in zone of inhibition for natural and synthetic antibiotics against *S. aureus***Table 9.** Results obtained from the ANOVA analysis for methanol and hexane extracts for *M. oleifera* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *P. aeruginosa*

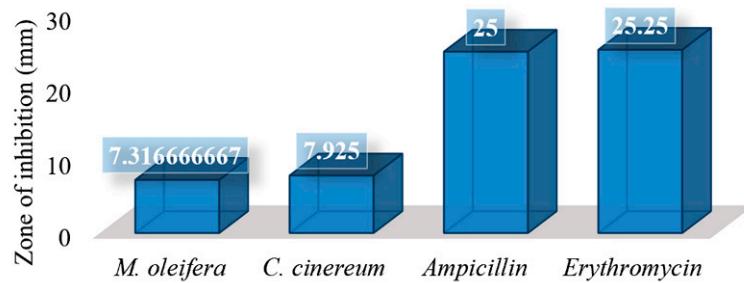
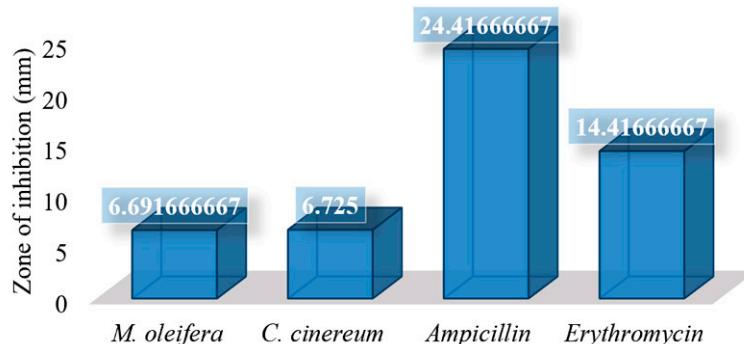
Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	287.2267	5	57.44533	6.525407	0.030121	5.050329
Within groups	19.25333	1	19.25333	2.18705	0.199236	6.607891
Error	44.01667	5	8.803333			
Total	350.4967	11				

Table 10. Mean and Standard deviation for methanol and hexane extracts for *C. cinereum* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *K. pneumoniae*

Concen.	% zone of inhibition (mm)	
	Methanol	Hexane
5	9.8±3.0	9.3±3.9
10	9.4±2.3	8.8±5.6
25	7.8±3.1	7.4±4.6
50	2.8±5.0	10.6±3.4
75	6.5±5.5	3.0±3.1
100	5.3±5.9	0.0±0.0

Table 11. Results obtained from the ANOVA analysis for methanol and hexane extracts for *C. cinereum* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *K. pneumoniae*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	69.7875	5	13.9575	1.383186	0.365277	5.050329
Within groups	0.520833	1	0.520833	0.051615	0.829274	6.607891
Error	50.45417	5	10.09083			
Total	120.7625	11				

**Fig. 8.** Difference in zone of inhibition for natural and synthetic antibiotics against *S. aureus***Fig. 9.** Difference in zone of inhibition for natural and synthetic antibiotics against *K. pneumoniae*

DISCUSSION

Plants with medicinal properties have the ability to heal and to cure many human associated diseases due to the presence of bioactive compounds, which is better known as phytochemical compounds/constituents (Wadood, et al., 2013). These phytochemicals add no nutrition value to the plant, in which it is found; thus it's also termed non-nutritive chemicals (Berardi, 2017). Phytochemicals occur naturally leaves, stem, roots, fruits and flowers of many plants; which when used individually or combined

Table 12. Mean and Standard deviation for methanol and hexane extracts for *M. oleifera* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *K. pneumoniae*

Concen.	% zone of inhibition (mm)	
	Methanol	Hexane
5	8.4±3.0	12.8±4.3
10	6.3±4.3	11.4±3.9
25	4.2±5.4	11.1±2.6
50	6.5±3.8	9.0±2.4
75	3.1±3.0	5.3±2.0
100	2.2±4.0	0.0±0.0

may safe guard the body against harmful bacteria (Wadood, *et al.*, 2013). These phytochemicals are divided into primary and secondary compounds; such as (chlorophyll, proteins and sugar) and (alkaloids phenolic saponins). The various phytochemical compounds whether primary or secondary exhibits important pharmacological abilities such as anti-viral, anti-inflammatory, anti-bacterial and even anti-cancer (Wadood, *et al.*, 2013). However, plants are more so enriched with the secondary compounds (Cowan, 1999), thus the reason why the secondary metabolites are more

Table 13. Results obtained from the ANOVA analysis for methanol and hexane extracts for *M. oleifera* at 100%, 75%, 50%, 25%, 10% and 5% concentration against *K. pneumoniae*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	118.8942	5	23.77883	4.815966	0.054747	5.050329
Within groups	29.7675	1	29.7675	6.028861	0.057555	6.607891
Error	24.6875	5	4.9375			
Total	173.3492	11				

Table 14. Correlation (Pearson) between the solvents and the concentration

Bacteria	Plant	Solvent	Correlation	Significance (0.707)
<i>S. aureus</i>	<i>C. cinereum</i>	Methanol	0.0133	No stat. sign.
		Hexane	-0.7840	Stat. sign.
	<i>M. oleifera</i>	Methanol	-0.6351	No stat. sign.
		Hexane	-0.9393	Stat. sign.
<i>P. aeruginosa</i>	<i>C. cinereum</i>	Methanol	-0.7244	Stat. sign.
		Hexane	-0.8928	Stat. sign.
	<i>M. oleifera</i>	Methanol	-0.9729	Stat. sign.
		Hexane	-0.8918	Stat. sign.
<i>K. pneumoniae</i>	<i>C. cinereum</i>	Methanol	-0.6824	No stat. sign.
		Hexane	-0.8344	Stat. sign.
	<i>M. oleifera</i>	Methanol	-0.8295	Stat. sign.
		Hexane	-0.9730	Stat. sign.

focused on for this research. So as this experiment was conducted, the first thing that was done, was figuring out if *C. cinereum* and *M. oleifera* have any phytochemicals. So the phytochemical analysis was done. The methanol extract of *C. cinereum* showed presence of alkaloids, catecholic tannins, saponins, flavones, volatile oils phenols and steroids; all of which have many beneficial properties including anti-bacterial properties. This would account for

the inhibitory effect seen in the extracts. These findings supports the research done by Cowan in 1999; where it was mentioned that plants are enriched with a variety of secondary metabolites such as those found above. It also corresponds to the findings of Guha *et al.*, 2009. Where they focused on "Therapeutic Potential of Polar and Non-Polar Extracts of Cyanthillium cinereum *In vitro*."

However, the methanol extract of *M. oleifera* only had alkaloids and steroids. The hexane extract of *C. cinereum* showed presence of Catecholic-tannins, saponins, flavones and steroids. However, the hexane extract of *M. oleifera* showed presence of only catecholic-tannins and steroids. They all have unique ways that aids in the destruction of microorganisms; some like saponins may affect the bacterial cell wall permeability, which in turn allow other phytochemicals such as flavones or flavonoids which have anti-inflammatory, antimicrobial to destroy the cell or its constituents (Jacob et al., 2009) and (Havesteen et al., 1983). Other phytochemicals; phenol among others, target the DNA of gram negative bacteria such as *K. pneumoniae* and *P. aeruginosa* thus destroying the cell/s (Cueva, 2010).

Antibiotics are medicines which has the ability to hinder the growth of or destroy microorganisms/microbes (Davis, 2017). The providence of antibiotics from phytochemicals such as those mentioned above, are obtained from natural sources and as such they are referred to as natural antibiotics. And as mentioned before they can be obtained from various plant parts (Chan, 2011). However, the phytochemicals along with other microorganisms are manufactured synthetically to be used as antibiotics and are referred to synthetic antibiotics. In other words they are laboratory-based chemical synthesis of natural sources (Chan, 2011). They are highly active, thus overuse and misuse can cause many serious side effects; such as antibiotic resistance, allergies and damage to vital organs. The natural antibiotics on the other hand, are safer as it relates to short and long term effects on the human body (Chan, 2011). Also the human body is inhibited with what is referred to as "good bacteria". Such bacteria have a mutualistic relationship our body. An example of such bacteria are *Lactobacillus johnsonii*, found in the gut and makes the process of milk digestion smoother. And such bacteria among others are constantly being harmed with the use of synthetic antibiotics, because as the antibiotics is administered either by rubs, oral, or injection, it has no regards as to whether the bacteria is beneficial or non-beneficial (Chan, 2011). In addition to the benefits of natural antibiotics, it not only kill or hinder the growth

of the bacteria but they also enhancing the body's natural capacity to fight off such bacterial infections in the future (Chan, 2011).

This research project took the form of a comparative study that incorporated the "Multi-factorial design", reason being the number of factors and parameters investigated. Such factors; three bacteria (*K. pneumoniae*, *P. aeruginosa* and *S. aureus*) treated with six concentrations (100%, 75%, 50%, 25%, 10% and 5%) extracted by two solvents (methanol and hexane) from the two selected plants (*Cyathillium cinereum* and *Moringa oleifera*). To facilitate main effect, interaction, and graph and statistical comparison of the multi-factor design (Piper, 2012), analysis such as ANOVA two-factor without replication, regression and correlation were done.

The effects of extracts from *C. cinereum* and *M. oleifera* were conducted using disk diffusion susceptibility method (Hudzicki, 2009). Comparison of the P-values of the extracts indicated that the extracts of *M. oleifera* had greater variation among the zones of inhibition compared to the extracts of *C. cinereum*. However, the *C. cinereum* hexane extract showed the largest zone of 22.1mm at 5% concentration against *S. aureus* as compared to the *M. oleifera* extracts that also showed the most effectiveness against *S. aureus* than the other two bacteria; this may be due to the presence of the phytochemicals found from the previous phytochemical analysis. The *M. oleifera*, on the other hand, was also most effective against the bacterium *S. aureus* than the other bacteria but only achieved an inhibitory zone of 14.9mm in diameter at 5% concentration.

It was indicated that as the concentration increase (5%-100%) the effectiveness of the extracts seems to decrease. This is due to three factors concentration (viscosity), solubility and particle size (Hudzicki, 2009). After the soaked disks were placed onto the selected agar (Mueller-Hinton is preferred for this type of experiment because it is not selective; any microorganism can grow) water moves from the agar into the disks (this maybe as a result of osmosis) and the antibiotics that is embedded in the disk diffuses through the agar (Hudzicki, 2009). However, the rate at which the antibiotic moves through the agar decreases as it moves further away from the source (disk); thus the concentration highest

in close proximity to the disk, this being due to the diffusion and molecular/particle size of the extract (Hudzicki, 2009). The third factor being the solubility properties of the extract also affects the zone of inhibition (Hudzicki, 2009). This explains not only the decrease in zone sizes but also explains why some hexane extracts showed not result at the higher concentrations (50%, 75% and 100%). The hexane evaporates very rapidly causing the extract to become thick thus decreasing the rate of diffusion at the higher concentrations of the crude extract.

The (Pearson) correlation analysis also supported the increase in concentration with the rate of diffusion. The purpose of running a correlation analysis is basically to outline the relationship between two continuous variables (Zou *et al.*, 2003). In this case the two variables are the concentration and zone of inhibition. Based on the correlation analysis, the results indicates that the concentration of the extract is indirectly proportional to the zone of inhibition. This is as a result of the correlation coefficient values obtained from the analysis being larger than that of the reference value which is 0.707. As long as the correlation coefficient value is larger than the reference/standard value (0.707) the more significantly correlated the values are and the difference in signs (positive or negative) indicates direction of the relationship (Zou *et al.*, 2003).

CONCLUSION

This study provides evidence that both *C. cinereum* and *M. oleifera* have antibiotic properties that can be used to hinder the growth of bacteria. The hexane extracts of *C. cinereum* had a statistically significant effect against the gram positive bacterium (*S. aureus*) than *K. pneumoniae*, *P. aeruginosa* (gram negative bacteria). Similarly, *M. oleifera* extracts was most effectiveness against *S. aureus* than the other two bacteria. As related to the potency of natural antibiotics, the *C. cinereum* was more effective against all three bacteria than the *M. oleifera*. However, based on overall susceptibility of the bacteria, Ampicillin followed by Erythromycin showed a significant potency against *S. aureus*, *K. pneumoniae* and *P. aeruginosa* compared to the potency of *C. cinereum* and *M. oleifera*.

ACKNOWLEDGMENTS

I would like to extend words of gratitude to GOD for the wisdom, strength and the provision of the many medicinal plants that was made available for us. I would also like to thank my family, Mrs. Ruth Daniel (Supervisor), Mr. Elford Liverpool, Prof. Abdullah Ansari, Mr. Yugraj Crepaull, Mr. Seon Homer, Mr. Randy (IAST/Food and Drug), and my friends.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

None.

AUTHORS' CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not Applicable.

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