International Journal of Agriculture and Animal Production
ISSN 2799-0907
Vol: 04, No. 04, June-July 2024
http://journal.hmjournals.com/index.php/IJAAP
DOI: https://doi.org/10.55529/ijaap.44.32.43

Effect of Moringa Oleifera Seed Extracts on Escherichia Coli

Thankyou Saturday Okpabi[1*], Uche Joy Nzube[2], Roberts Chioma Lilia[3]

[1,2,3]Biology Department, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rumuolumeni Port Harcourt, Rivers State, Nigeria.*

Email: [2]spectabilisjoy70@gmail.com, [3]liliachioma@gmail.com
Corresponding Email: [1]okpabis@gmail.com*

Received: 02 February 2024 **Accepted:** 19 April 2024 **Published:** 03 June 2024

Abstract: ***This work is to determine the effect of wonder plant (Moringa oleifera) seed extracts on Escherichia coli. E. coli was isolated, characterized and identified. The moringa seed extract was extracted and characterized, concentration was prepared, preparation of sensitivity disc with the extract, determination of minimum inhibitory concentration and minimum bacterial concentration of extract were done while antibiotics susceptibility test served as the control. The results show that ethanol/extract was highly effective followed by hot water/extract while cold water/extract was the least as the P>0.5. The extracts show promise as a natural antibacterial agent that could be used to combat microorganisms responsible for waterborne illnesses.***

Keywords: Moringa Oleifera, Seed Extract, Escherichia Coli.

1. INTRODUCTION

Infections caused by Escherichia coli range from mild gastrointestinal discomfort to severe and potentially life-threatening conditions. The dangers of *E. coli* infections depend on the specific strain of the bacterium and the individual's health status. Most E. coli infections are associated with gastroenteritis, causing symptoms such as diarrhoea, abdominal cramps, nausea, and vomiting (Walter et al, 2011). Certain strains of E. coli, such as E. coli O157:H7, produce toxins that cause a severe complication known as haemolytic uremic syndrome (HUS), characterized by haemolytic anemia, thrombocytopenia (low platelet count), and acute kidney failure. Some strains of E. coli cause urinary tract infections, leading to discomfort and pain, and, if left untreated, could potentially spread to the kidneys, causing more serious complications. In certain cases, E. coli infections progress to systemic infections, causing sepsis, a life-threatening condition where the body's response to infection leads to widespread inflammation and organ failure (Jennifer & Ancharna, 2014). Contaminated food and water can be sources of E. coli infections. Certain strains of E. coli have acquired antibiotic resistance,

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which complicates the treatment of illnesses. Consequently, this results in an extended period of sickness, escalated medical expenses, and an elevated likelihood of developing other difficulties (Zunio, 2015).

2. RELATED WORKS

In their study, Lar et al. (2011) found that "Moringa oleifera effectively suppressed the growth of all seven tested microorganisms, including *Escherichia coli, Staphylococcus aureus, Salmonella Typhi, Pseudomonas aeruginosa, Shigella flexneri,* and *Proteus vulgaris*.
Oliveira & Silvera (1999) conducted a study on Moringa oleifera, in which they analysed its chemical composition, antimicrobial activity, and antioxidative activity. They found that Moringa oleifera exhibited notable activity against various bacteria, *including Acinetobacter calcoaceticus, Beneckea natriegens, Citrobacter freundii, Erwinia carotovora, Lactobacillus plantarum, Micrococcus luteus,* and *Staphylococcus aureus*.
Zunio et al (2015) provided evidence of the efficacy of extracts derived from Moringa oleifera, Acrous calamus, Ocimum sanctum, Zingiber officinale, and Cinnamomum zeylanicum. Moringa oleifera and Piper betle shown antibacterial effectiveness against S. aureus, E. coli, P. aeruginosa, and Klebsiella species, with E. coli displaying greater resistance to the extract.
In their study, Sutherland et al (2001) examined the antibacterial properties of Moringa oleifera against E. coli, S. aureus, Bacillus cereus, and Salmonella typhimurium. They discovered that Moringa oleifera was efficacious in combating all four bacteria: E. coli, S. aureus, B. cereus, and Salmonella typhimurium.
In a study conducted by Santosh (2013), the antimicrobial properties of separate and combined portions of dill, cilantro, Moringa oleifera, coriander, and eucalyptus oils were investigated. It was discovered that combining these fractions resulted in either additive, synergistic, or antagonistic effects against the microorganisms being tested. Notably, the distilled fractions of coriander oils exhibited significantly greater potency compared to the crude oil.
Oludora et al (2012) conducted an analysis on the antimicrobial properties of Moringa oleifera, garlic, ginger, clove, black pepper, and ground green chilli, as well as their aqueous extracts, against human pathogenic bacteria. The study revealed that clove completely eradicated yeast within a span of 5 hours, and it also moderately hindered the growth of Shigella flexneri.
Oliveira & Silvera (1999) conducted an analysis on the antimicrobial properties of black pepper, clove, geranium, nutmeg, oregano, and thyme against 25 different genera of bacteria. The study found that black pepper and Moringa oleifera extracts were highly effective, completely eliminating colonies of S. aureus, B. cereus, and B. subtilis strains of bacteria at levels of 5 and
In their study, Nepolean et al. (2019) discovered that Moringa oleifera had the highest efficacy across all tested strains, including S. aureus, K. pneumoniae, P. aeruginosa, E. coli, Enterococcus faecalis, Mycobacterium smegmatis, Micrococcus lutius, and Candida albicans. S. aureus and C. albicans were the bacteria most vulnerable to the effects of cinnamon. Pseudomonas aeruginosa and Escherichia coli had the highest level of resistance among all the microorganisms tested against the spice sample.
Auwal et al (2013) assessed the antimicrobial efficacy of six Indian spice extracts, specifically clove, cinnamon, mustard, Moringa oleifera, ginger, and mint, against potent foodborne

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pathogens, namely E. coli, S. aureus, and B. cereus. The study revealed that clove and mustard extracts exhibited significant inhibitory effects at a concentration of 1%. Additionally, cinnamon leaf volatile oil and oleoresin demonstrated superior inhibitory outcomes compared to bark oils". The aim was to ascertain the effects of *Moringa oleifera* seed extract on Escherichia coli. The precise objectives were to:

i. isolate and identify *Escherichia coli*;
ii. analyze the phytochemical properties of *Moringa oleifera* seed extract;
iii. determine the antibiotic susceptibility reaction of the extract on *Escherichia coli* compared with standard antibiotics.

3. RESEARCH METHODOLOGY

Isolation and Identification of *Escherichia Coli:* The test organism was extracted from a student's faeces. The faeces were collected in a sterile container and homogenized and 1 ml from the stock was used for serial dilutions. The pour-plate method and streaking techniques were used to isolate the bacterium present. One miililiter (1 ml) of the dilutions 10^4, 10^3, 10^2, and 10^1, were pipetted into nutrient agar plates and blood agar. The isolate obtained was identified using colonial morphology and oil immersion lens (x 100) and the plate was incubated in triplicates aerobically and anaerobically at 37° for 24 hours. Later, discrete colony were sub-cultured on fresh medium for the development of pure isolate, which was stored on nutrient Agar slants for biochemical test (gram's stain, oxidase, coagulase, catalase, indole, citrate, sugar fermenters, MR-VP, motility).

Phytochemical Analysis of *Moringa Oleifera*
The fruits were bought at mile 3 pack Port-Harcourt, Rivers State and dried in the shade for three weeks. They were then crushed into a powdered form using a clean and sterile electric blender. A quantity of four grammes (4g) of the powdered fruit was immersed in thirty millilitres (30 ml) of cold water, hot water, and ethanol. Every conical flask was sealed with cotton wool that was wrapped in aluminium foil and placed in a rotary shaker for a duration of two days. The extracts underwent filtration using a sterile filter paper (Whattman No 1). The liquid samples were subjected to evaporation until all the solvent had completely evaporated, utilising a water bath maintained at a temperature of 35oC for a duration of two days.

Phytochemical analysis (Qualitative)
Flavonoid (Alkaline reagent test): A precisely measured volume of 0.2 ml of the extracts was subjected to treatment with six drops of a 2% sodium hydroxide solution. The appearance of a vibrant yellow hue, which subsequently fades to a colourless solution upon the introduction of a weak acid, serves as evidence for the existence of flavonoids in the extract.
Alkaloids (Mayer's test): 0.5 ml of the extracts, weighing one half gramme, were dissolved in 5 ml of a 1% solution of hydrochloric acid that had been diluted, and subsequently filtered. The filtrate underwent treatment with Mayer's reagent, which is composed of Potassium mercuric iodide. A favourable indication for the presence of alkaloids in the extracts is observed when a yellow-colored precipitate is formed.

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Terpenoids (Salkowski's test): Precisely 0.1 ml of the extracts were combined with 0.5 ml of chloroform, and then 1 ml of strong sulphuric acid was added. The occurrence of a reddish-brown solid formed as a result of a chemical reaction signifies the existence of terpenoids in the extracts.
Tannins (Ferric chloride test): A precisely measured amount of the extract (0.2ml) was combined with an equal proportion of distilled water in a test tube. Subsequently, three drops of diluted ferric chloride were introduced. The development of a brownish blue or dark hue served as a sign of the presence of tannins in the extracts.
Steroids (Liebermann-Burchard's test): The extracts (0.5 ml) were combined with 2 ml of chloroform. The liquid in a test tube was supplemented with 2 ml of concentrated sulphuric acid. A positive outcome for steroids in the extracts was shown by the presence of a red colour in the lower chloroform layer.
Saponins (Foam test): A precisely measured quantity of 0.2 ml of the extracts was combined with 6 ml of distilled water in a graduated cylinder. The mixture was violently agitated for a duration of 15 minutes. The occurrence of bubbles or long-lasting foam for a duration of 10 minutes will serve as an indication of the existence of saponins in the extracts.
Phenols (Ferric chloride test): Exactly 0.2 millilitres of the extracts will be added, along with 2 millilitres of a 5% aqueous ferric chloride solution. Phenols in the extracts yield a good result indicated by the production of a bluish hue.
Cardiac glycoside test: Precisely two millilitres (2.0 ml) of the extracts were dissolved in an equal volume of chloroform. Subsequently, two millilitres (2 ml) of sulphuric acid were added cautiously and the mixture was gently agitated. The presence of a steroidal ring can be inferred from a reddish-brown coloration.

Quantitative Phytochemical Analysis
Determination of Total Favonoid Content: The quantification of flavonoids in Moringa oleifera seed extracts was conducted using "the aluminium chloride colorimetric method, with quercetin serving as the standard. The flavonoid concentration was expressed as quercetin equivalent. The concentrations of 10, 25, 50, 75, and 100µg/ml were produced in methanol using the standard quercetin solution. Each quercetin dilution, measuring 100µl, was combined with 500 µl of distilled water, followed by the addition of 100µl of 5% sodium nitrate. The mixture was then left undisturbed for a duration of 6 minutes. Next, 150µl of a 10% solution of aluminium chloride was introduced and left undisturbed for 5 minutes. Subsequently, 200µl of a 1M solution of sodium hydroxide will be added in a sequential manner. The measurement of absorbance in this reaction mixture was conducted at a wavelength of 510nm using a single-beam UV-VIS spectrophotometer (UV mini-1240). The quantification of total flavonoids content was achieved by utilising a linear equation derived from a standard curve generated with quercetin. The results were reported as milligrammes of quercetin equivalent (QE) per gramme of dry extract, following the methodology described by Khandelwal in 2001".

Determination of Total Phenol Content: The Folin-Ciocalteu reagent was employed to quantify the total phenol content of Moringa oleifera seed extracts, with gallic acid serving as the reference standard for constructing the calibration curve. A 0.5 ml portion of gallic acid solutions with concentrations of 10, 20, 40, 80, and 100µg/ml were combined with 2ml of

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Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and then neutralised with 4ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was left at room temperature for 30 minutes with periodic agitation to facilitate the development of colour. The intensity of the produced blue colour was quantified at a wavelength of 765 nm using a single-beam UV-VIS spectrophotometer (UV mini-1240). "The total phenols content was quantified using a linear equation derived from a standard curve generated using gallic acid. The results were represented as milligrammes per gramme of dry extract, equivalent to gallic acid (GAE) (Khandelwal, 2001)".

Determination of Total Saponin Content: A precisely measured volume of 5000µL of water was combined with a precisely measured volume of 100µL of diosgenin. 500 microliters of vanillin reagent (8 grammes of vanillin dissolved in 100 millilitres of 99.5% ethanol) was added. An additional 5 cc of sulphuric acid with a concentration of 72% was added and thoroughly mixed. The solution was incubated in a water bath at a temperature of 60°C for a duration of 10 minutes. After a duration of 10 minutes, the substance underwent a cooling process, following which the absorbance was measured at a wavelength of 544nm and subsequently documented. The total saponins content was quantified using a linear equation derived from a standard curve generated using diosgenin. The results were represented as milligrammes of diosgenin equivalent (DE) per gramme of dry extracts.

Determination of Total Alkaloid Content: 1 millilitre of unrefined extracts from Moringa oleifera seeds were measured and placed in a 100 millilitre container. Then, 50 millilitres of a solution containing 10% hydrochloric acid in ethanol were added to the container. The container was covered and left undisturbed for a duration of 4 hours. The solution was passed through a filter, and the resulting liquid was then heated to 78 degrees Celsius and reduced to one-fourth of its initial volume using a rotary evaporator. The precipitation was completed by adding 15 drops of concentrated ammonium hydroxide drop by drop to the concentration. Following a 3-hour period of mixture sedimentation, the liquid portion above the sediment was discarded, and the solid particles were rinsed with 20 ml of a solution containing 0.1M ammonium hydroxide. The resulting mixture was then passed through a filter. The remaining substance was an alkaloid, which was dried and measured for weight. The percentage alkaloid was determined by dividing the weight of the residue by the weight of the sample obtained, and then multiplying by 100 (Khandelwal, 2001).

Determination of Tannin: 0.5 grammes of the ground sample were dissolved in 50 millilitres of distilled water, which was placed in a 250 millilitre conical flask. The mixture was agitated using a magnetic stirrer for a duration of 1 hour, and thereafter, 5 ml of the resulting liquid was transferred into a 50 ml volumetric flask. 0.1 grammes of tannic acid were dissolved in 100 millilitres of distilled water. Then, 5 millilitres of the resulting tannic acid solution were transferred into a 50 millilitre volumetric flask using a pipette. A blank specimen was established by utilising 5 ml of distilled water. A volume of 1 ml of Folin-Dainas reagent was applied to each sample, followed by the addition of 2.5 ml of a saturated solution of sodium carbonate (Na2CO3). A volume of 50 ml of distilled water was introduced to dilute the mixture. Subsequently, the spectrophotometer was used to measure the absorbance of the resulting black

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colour after incubating at a temperature of 25°C for a duration of 90 minutes. The measurement of absorbance was conducted at a specific wavelength of 760 nm, using a reagent blank set at zero as a reference (Khandelwal, 2001).

Determination of Cyanogenic Glycosides: A colorimetric approach utilising alkaline picrate was employed. A volume of 1 millilitre (1 ml) of the processed Moringa oleifera seed extracts was diluted in 150 millilitres (150 ml) of distilled water in a conical flask. A standard cyanide solution of 1 ml was made using the same method. A strip of picrate paper with alkaline properties was placed above the mixture and secured using a rubber stopper. Precautions were taken to prevent the paper from making contact with the surface of the mixture. The setup was let to remain undisturbed for a duration of 18 hours, specifically during the nighttime, at the ambient temperature of the room. A total of 60 cc of distilled water was employed to elute the picrate sheets on the subsequent day. The absorbance was measured using a spectrophotometer at a wavelength of 540 nm, with a reagent blank used as a reference (Khandelwal, 2001).

Concentration of Extracts
Lower concentrations were obtained by dilution of the extracts to obtain concentrations of 100mg/ml, 75mg/ml, 50mg/ml, and 25mg/ml. The tubes carrying the different concentrations were clearly marked, and they were used right away.

Preparation of Sensitivity Discs with Extracts
Using a cork borer, 6mm-diameter discs were punched out and placed in a sterile glass petri dish. The discs were then sterilized at 170°C for approximately 30 minutes, after which they were allowed to cool. The varied extract concentrations were applied to the already sterilized paper discs. 12 discs total were submerged in each concentration. The solution was given to the discs to absorb.

Antibacterial Assay Using Disc Diffusion Method
The disc diffusion technique, first described by Kirby-Bauer in 1966, was used for this. In sterilized petri dishes, Mueller Hinton agar medium was added and allowed to set. On the solidified agar, ten (10) mls of the test organisms' 0.5 McFarland standards were poured and evenly distributed. Using sterile forceps, the wet discs were picked up and placed on the agar's surface. At 37 °C, the plates were incubated for 24 hours. A meter rule was used to measure the clearance zone. Controls were set up with discs soaked in ethanol, hot and cold water, and various solvents, but without plant extract (Jennifer & Anchana, 2014).

Determination of Minimum Inhibitory Concentration of the Extracts
Using the Broth Dilution Method, the lowest inhibitory concentration of the plant extracts was established. 100 mg/ml and 75 mg/ml of the diluted extract were present in one (1.0) ml. 9ml of Mueller Hinton broth were combined with 50mg/ml and 25mg/ml in several test tubes. After everything had been well combined, 0.1ml of the test isolates' 0.5 McFarland standards was added to the tubes as an inoculant. After 24 hours of incubation at 37°C, the tubes were checked for turbidity. In each example, the MIC was defined as the lowest dose of extract that prevented

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the inoculated test isolates from exhibiting turbidity in the broth medium. As controls, test tubes that were inoculated with the test isolates but not the extracts were used.

Determination of Minimum Bacterial Concentration of the Extracts
The extracts with undetectable growth were cultured on Mueller-Hinton agar plates and incubated at 37oC for 24 hours. The minimum bactericidal concentration was determined as the lowest proportion of the extracts that exhibited no growth on the plate after 24 hours.

Antibiotic Susceptibility Test
A 0.5ml broth culture of the standardized test organisms was swabbed onto the dried surface of the Mueller Hinton agar plate. "The inoculated agar plate was covered with commercially available antimicrobial discs, and the plates were incubated at 37°C for 24 hours" (Jabeen et al. 2018).

4. RESULT AND DISCUSSION

Table 4.1: Biochemical test and the probable organism

characterization	Indole	MR	VP	Citrate	Gram Stain	Catalase Test	Motility Test	Oxidase Test	Coagulase Test	S/F	Shape	Probable Genus
Isolate	+	+	-	-	-	+	+	-	-	+	rod	*E. coli spp*

Table 4.1 shows that the combination of these test results, including the positive indole test and rod shape, aligns with the characteristics commonly associated with Escherichia coli species. E. coli is a Gram-negative, rod-shaped bacterium that is catalase-positive, oxidase-negative, and typically non-motile. the isolate appears to exhibit characteristics consistent with Escherichia coli species based on the results of the provided biochemical tests.

Table 4.2: Physiochemical Composition of the *Moringa oleifera* extract

Phytochemical Constituent	Relative Presence	Ethanol g/kg	Hot Water g/kg	Cold Water g/kg
Alkaloid	+ + +	0.360± 0.01	0.210±0.01	0.107±0.01
Cardiac glycoside	+	0.002± 0.00	0.001±0.00	0.000±0.00
Flavonoid	+	0.073±0.00	0.052±0.00	0.031±0.00
Phenol	+ +	0.393±0.02	0.218±0.02	0.1190.02
Saponin	+ + +	4.990±0.15	3.190±0.15	2.101±0.15
Steroid	+ + +	4.110±0.13	3.171±0.13	2.610±0.13
Tannin	+ +	0.451±0.02	0.220±0.02	0.110±0.02
Terpenoid	+ + +	2.194±0.03	1.860±0.03	0.671±0.03

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Table 4.3: ANAOVA Table

SV	df	SS	MSS	F_{cal}	F_{tab}
Treatment	2	27.49	13.75	3.65	2.93
Residual	21	79.16	3.77		

Table 4.2 revealed the presence of saponin (4.990, 3.190, 2.101±0.15), tannin (0.451, 0.220, 0.110±0.02), cardiac glycoside (0.002, 0.001, 0.000± 0.00), flavonoid (0.073, 0.052, 0.031±0.00) and alkaloid (0.360, 0.210, 0.107±0.01), Phenol (0.393, 0.218, 0.119±0.02), Terpenoid (2.194, 1.860, 0.671±0.03), and Steroid (4.110, 3.171, 2.610±0.13) were respectively observed. The extracts were rich in saponins, steroids, and terpenoids, suggesting potential bioactive compounds with various biological activities. The presence of alkaloids, phenols, and tannins in moderate concentrations indicates a diverse phytochemical profile while table 4.3 shows no significance difference among the extracts as the Fcal (3.65) was more than the Ftab (2.93) at 5% degree of freedom.

Table 4.4 showing the antibacterial activity of cold aqueous, hot aqueous, and ethanoic extracts of seeds of *Moringa oleifera* on test on *Escherichia coli*

Concentration of *M.oleiferna* seed extract (mg/m)	Inhibition zone distribution			
	Hot water	**Cold water**	**Ethanoic**	**Control (ofloxacin)**
100	11	10	13	19
75	10	9	11	18
50	8	6	11	15
25	6	5	10	13
Mean	8.75	7.5	11.0	16.25

Table 4.4 shows that the zones of inhibition for concentrations 100, 75, 50, and 25 mg/mL for hot water extract are 11, 10, 8, and 6 mm, the zones of inhibition for concentrations 100, 75, 50, and 25 mg/mL for cold water extract are 10, 9, 6, and 5 mm, the zones of inhibition for concentrations 100, 75, 50, and 25 mg/mL for ethanoic extract are 13, 11, 11, and 10 mm, while the zones of inhibition for concentrations 100, 75, 50, and 25 mg/mL for the control are 19, 18, 15, and 13 mm, respectively.

Table 4.6: Showing Inhibition Zone

	N	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
					Lower Bound	**Upper Bound**		
Hot water	4	8.7500	2.21736	1.10868	5.2217	12.2783	6.00	11.00
cold water	4	7.5000	2.38048	1.19024	3.7121	11.2879	5.00	10.00

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Ethanol	4	11.2500	1.25831	.62915	9.2478	13.2522	10.00	13.00
Anti-biotic	**4**	**16.2500**	**2.75379**	**1.37689**	**11.8681**	**20.6319**	**13.00**	**19.00**
Total	16	10.9375	3.99114	.99778	8.8108	13.0642	5.00	19.00

The table above showed that ethanol concentration is second higher concentration (11.25±1.26), followed by hot water (8.75±2.22) while the concentration of M. oleifera in cold water is the least (7.50±2.30). none of the concentration was higher that of the anti-biotic (16.25±2.75).

4.7 ANOVA Table

SV	df	SS	MSS	F_{cal}	F_{tab}
Conc. Extract Error	3 3 9	53.2 179.8 5.9	17.73 59.93 0.66	26.86 90.80	3.86 3.86

At 0.5% level of significance
Sine the P-value is >0.5, it means that we accept the alternate hypothesis which says, there are significance difference in the effects of the four treatments, that is the extract (hot water, cold water, ethanoic) and the control(ofloxacin) at 5% level of freedom. Both the extracts and the antibiotic do not have similar inhibition effects on the bacterium (Escherichia coli).
Table 4.1 shows that the combination of these test results, including the positive indole test and rod shape, aligns with the characteristics commonly associated with Escherichia coli species. E. coli is a gram-negative, rod-shaped bacterium that is catalase-positive, oxidase-negative, and typically non-motile. The isolate appears to exhibit characteristics consistent with *Escherichia coli* species based on the results of the provided biochemical tests. This result tallies with that of Zunio et al (2015) & Vinoth et al (2012), as the isolate (the bacterial sample being tested) appears to exhibit characteristics consistent with Escherichia coli species. The results suggest that the bacterial isolate in question is likely Escherichia coli based on its positive indole test, rod-shaped morphology, and alignment with other characteristic features associated with this species.
Table 4.2 revealed the presence of saponin (4.990, 3.190, 2.101±0.15), tannin (0.451, 0.220, 0.110±0.02), cardiac glycoside (0.002, 0.001, 0.000± 0.00), flavonoid (0.073, 0.052, 0.031±0.00) and alkaloid (0.360, 0.210, 0.107±0.01), phenol (0.393, 0.218, 0.119±0.02), terpenoid (2.194, 1.860, 0.671±0.03), and steroid (4.110, 3.171, 2.610±0.13) were respectively observed. The extracts were rich in saponins, steroids, and terpenoids, suggesting potential bioactive compounds with various biological activities. The presence of alkaloids, phenols, and tannins in moderate concentrations indicates a diverse phytochemical profile, while table 4.3 shows no significant difference among the extracts as the Fcal (3.65) was greater than the Ftab (2.93) at 5% degree of freedom. This result corresponds with the findings of Anwar & Rashid (2017); Jennifer & Anchana (2014); Odebiyi & Sofowora (2018) who added that the extracts are particularly rich in saponins, steroids, and terpenoids, suggesting that these compounds are

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present in relatively higher concentrations compared to others because the presence of saponins, steroids, and terpenoids in the extracts suggests the potential for bioactive compounds, and these substances are known for their diverse biological activities, which could include medicinal properties or other beneficial effects, and also admitting that the extracts have a diverse phytochemical profile. This diversity is indicated by the presence of alkaloids, phenols, and tannins in moderate concentrations, as a diverse phytochemical profile is often desirable as different compounds contribute to various health benefits.

Table 4.4 shows that the zones of inhibition for concentrations 100, 75, 50, and 25 mg/mL for hot water extract are 11, 10, 8, and 6 mm, the zones of inhibition for concentrations 100, 75, 50, and 25 mg/mL for cold water extract are 10, 9, 6, and 5 mm, the zones of inhibition for concentrations 100, 75, 50, and 25 mg/mL for ethanoic extract are 13, 11, 11, and 10 mm, while the zones of inhibition for concentrations 100, 75, 50, and 25 mg/mL for the control are 19, 18, 15, and 13 mm, respectively. If the P-value is >0.5, it means that we accept the alternate hypothesis, which says there are significant differences in the effects of the four treatments, namely the extract (hot water, cold water, ethanoic) and the control (ofloxacin) at 5% level of freedom. Both the extracts and the antibiotic do not have similar inhibition effects on the bacterium (Escherichia coli). This result agrees with findings of Zunio et al (2015) and Vinoth et al (2012). The result suggests that the extracts and the antibiotic operate through different inhibition mechanisms because different extracts may show varying degrees of effectiveness against *Escherichia coli*. This information is valuable for potential drug development or the formulation of natural antimicrobial agents. This indicates a diverse set of active compounds or substances in the extracts compared to the antibiotic. The extracts, which likely contain phytochemicals from the plant source, demonstrate varied effects on *Escherichia coli*. The diversity in the composition of phytochemicals in the extracts might be responsible for the observed differences in inhibition effects. The finding suggests that combining extracts with the antibiotic may offer a synergistic or additive effect, targeting the bacteria through multiple mechanisms, because combinatorial therapies have the potential to enhance the overall antibacterial efficacy and reduce the risk of antibiotic resistance. The result highlights the complexity and diversity of the inhibitory effects of different extracts and antibiotics on *Escherichia coli*. This information guides further research, including the identification of active compounds, exploration of combinatorial therapies, and consideration of specific applications for different extracts. Since the p-value is greater than 0.5, it suggests that there is no significant difference between the effects of the extracts and the control on *Escherichia coli*, meaning the alternate hypothesis, which states that there is a significant difference, is accepted.

5. CONCLUSION

This study has successfully shown that seed extracts of *M. oleifera* have antimicrobial properties against *E. coli.* The utilisation of extracts offers an economical and enduring approach to mitigate diseases, thereby enhancing the standard of living for those residing in both rural and urban areas of developing nations. The extracts' ability to inhibit can be linked to the presence of specific phytochemical ingredients in them.

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ISSN 2799-0907
Vol: 04, No. 04, June-July 2024
http://journal.hmjournals.com/index.php/IJAAP
DOI: https://doi.org/10.55529/ijaap.44.32.43

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International Journal of Agriculture and Animal Production
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