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THE ANTIMICROBIAL ACTIVITY OF n-C₆H₁₄ AND CH₃CH₂OH EXTRACT OF *SOLANUM MELONGENA* FRUIT AND LEAVES OF *MORINGA OLEIFERA*

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ABSTRACT: As part of a research initiative to evaluate plants used for their nutritional and herbal values, the antimicrobial activity of *Solanum Melongena*'s fruit and *Moringa oleifera*'s leaves were investigated. Each plant part was subjected to selective extraction using solvents of varying polarity: n-C₆H₁₄, CH₂Cl₂, EtOAc and CH₃CH₂OH. The n-C₆H₁₄ and CH₃CH₂OH extract of these two plants were tested for their antimicrobial activity at three different concentrations of 5%, 10% and 20% of crude extracts against three pathogenic bacterial strains: *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* using the Disc diffusion assay. Both n-C₆H₁₄ and CH₃CH₂OH extracts showed antibacterial activity at a higher concentration of 20% of crude extract. The order of bacteria susceptibility to *Moringa oleifera* extract been *S. aureus* > *K. pneumoniae* > *E. coli* whereas that for *Solanum Melongena* extract been *S. aureus* > *E. coli* > *K. pneumoniae*. The area of zone of inhibition ranges from 44.15 mm² to 53.55 mm². These investigations suggest that the extracts of *Moringa oleifera* and *Solanum Melongena* can be used as antibacterial agents in addition to their nutritional value.

INTRODUCTION: Guyana has a rich bio diversified flora whose organic and aqueous extract have been shown to possess potent and selective antimicrobial activity compared with standard antibiotics such as penicillin, nystatin and ampicillin¹⁻⁷ etc. Research in the design and syntheses of antimicrobials will be everlasting endeavours on our planet considering the fact that bacteria and fungus developed resistance to antimicrobials over a period of time⁸⁻¹³. Antibiotic resistance has become a global concern¹³. This is primarily due to indiscriminate use of commercial antimicrobial drugs used for the treatment of infectious diseases.

This has led to the search for new antimicrobials, both herbal and synthetic.

However, synthetic drugs/medicine has several adverse side effects which are usually irreversible when administered and the cost of synthesizing drugs in most cases is an expensive endeavour¹⁰⁻¹². In addition, phytochemical screening and natural products isolation can lead to novel and know natural products whose *in vitro* antimicrobial activity can be correlated with that of the crude plant extract¹⁴⁻¹⁵.

There is also a need to assess the medicinal values of plant used as food source. Thus, efforts should be made to intensify the production of food crops in the agro-industry that have antimicrobial properties in addition to their nutritional properties. As such, the antimicrobial activity of the n-C₆H₁₄ and CH₃CH₂OH extract of *Solanum melongena* (Solanaceae) and *Moringa oleifera* (Moringaceae) were investigated in

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vitro against pathogenic *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Such an endeavour will be a boost to the agro industry and Health Sector.

Moringa oleifera is the most widely cultivated species of the genus *Moringa*, the only genus in the Moringaceae. This plant is rich in unique compounds such as glucosinolates and isothiocyanates. Natural products such as 4-(4'-O-acetyl- α -L-rhamno

pyranosyloxy)benzyl isothiocyanate, 4-(α -L-rhamno pyranosyloxy)benzyl isothiocyanate, niazimicin, pterygospermin (2), benzyl isothio cyanate (1) and 4-(α -L-rhamnopyranosyloxy)benzyl glucosinolate isolated from *Moringa* species have been reported to have hypotensive and anticancer activity. Phytochemicals such as the carotenoids (β -carotene or pro-vitamin A) have also been isolated^{16, 17}. The structures of two of these compounds are shown in **Fig. 1**.

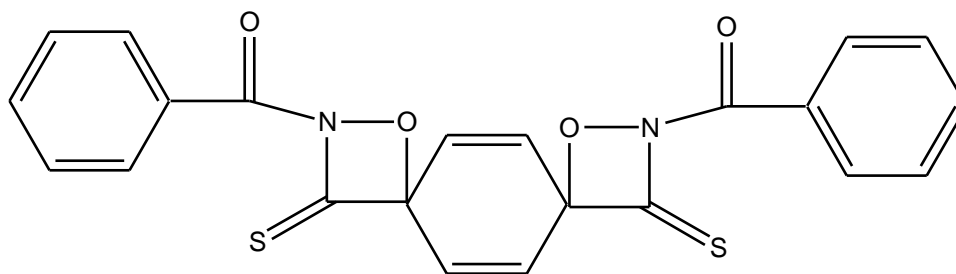
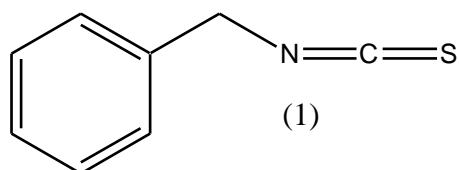


FIG. 1: BENZYL ISOTHIOCYANATE (1) AND PTERYGOSPERMIN (2) FROM MORINGA OLEIFERA

The leaves are the most nutritious and contain significant amount of vitamin B₆, vitamin C, provitamin A, β -carotene, magnesium and protein. Calcium in *Moringa oleifera* leaves are usually complexed as crystals of calcium oxalate.

Moringa oleifera provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals such as hexadecanoic acid, ethyl palmitate, palmitic acid, ethyl ester, 2,6-Dimethyl-1, 7-octadiene-3-ol, 4-Hexadecen-6-yne, 2-hexanone, and 3-cyclohexyliden-4-ethyl - E2-Dodecenylacetate¹⁷.

It is very important for its medicinal value. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess anti-tumour, antipyretic, anti-epileptic, anti-inflammatory and anti-ulcer activity¹⁷.

Moringa oleifera preparations have been used for its anti-trypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypocholesterolemic, and hypoglycemic activities, as well as having considerable efficacy in water purification by flocculation, sedimentation, antibiosis and even reduction of Schistosome cercariae titer¹⁸.

A new biflavonol glycoside, Solanoflavone was isolated from aerial part of *Solanum melongena*. The chemical structure was elucidated as isorhamnetin-3-O-beta-D-glucopyranoside-(4'->O->4''')-galangin-3''-O-beta-D-glucopyranoside on the basis of physico-chemical and spectroscopic methods, including 2D NMR spectral techniques¹⁹.

Flavonoids were isolated from *Solanum melongena* and shown to have potent antioxidant activity. Concentrations of malondialdehyde, hydroperoxides and conjugated dienes were lowered significantly²⁰.

Phenylethyl cinnamides as potential alpha-glucosidase inhibitors were isolated from the roots of *Solanum melongena* (Solanaceae). Bioassay-guided fractionation against alpha-glucosidase resulted in isolation and identification of six phenolic compounds from the 70% EtOH extract of the roots. Three of the phenylethyl cinnamides, N-transferuloyl tyramine, N-*trans*-p-coumaroyl tyramine and N-*cis*-p-coumaroyl tyramine possessed inhibitory activity against alpha-glucosidase with IC₅₀ values of 500.6, 5.3 and 46.3 microM, respectively. Mechanistic studies revealed these phenylethyl cinnamides as non-competitive inhibitors. The above is the first study of the alpha-glucosidase inhibitory activities of the roots of *Solanum melongena*, suggesting potential medicinal use of this herb²².

Phytochemical examination of the methanolic and aqueous extracts of the fruit and crown of *Solanum Melongena* showed the presence of alkaloids, saponins, steroids, tannins/ phenolics, flavonoids, proteins and carbohydrates. Ascorbic acid and phenolics both which are powerful antioxidants were present in fruit, the presence of saponins and glycoalkaloids which were also found in the fruit protects plant from microbial pathogens.

Various parts of *Solanum melongena* (Solanaceae) are useful in the treatment of inflammatory conditions, cardiac debility, neuralgia, ulcers of nose, cholera, bronchitis and asthma. Roots are used as anti-asthmatic and general stimulant, juice is employed for otitis, applied to ulcers of the nose. Leaves are used in the treatment of bronchitis, asthma and dysuria, also given in liver complaints and they stimulate inter hepatic metabolism of cholesterol. The fruit of *Solanum melongena* is a high valued vegetable all over the world because of its taste and higher percentage of Vitamin B₂. The fruit is also used in the treatment of diabetes²².

MATERIALS AND METHODS:

Reagents and materials: Antibiotics, Ampicillin, Mueller Hinton Agar, agar plates were purchased from the International Pharmacy Association (IPA) in Guyana. Bacterial culture was obtained from the Georgetown Public hospital.

Collection of Plant material: Fresh leaves of *Moringa oleifera* and fruits of *Solanum meoligena* were handpicked at Friendship village on the Corentyne, Berbice, and placed in bags. These were washed with tap, distilled water and were dried for four (4) hours. They were further air dried for one week and sent for authentication at the Centre for the Study of Biological Diversity, University of Guyana.

Grinding and Extraction: Approximately six hundred grams (600 g) of the fruit of the *Solanum melongena* were cut into small pieces and blended thrice in six hundred milliliters (600 ml) of n-C₆H₁₄. The contents were then filtered into air tight glass containers. The procedure was repeated using the more polar CH₃CH₂OH solvent. Leaves of *Moringa oleifera*, 600 grams in weight was also blended thrice in 600 ml of n-C₆H₁₄. The contents were filtered into air tight glass containers. The procedure was repeated using freshly distilled CH₃CH₂OH. The contents for each extraction was filtered, solvents dried over anhydrous Na₂SO₄ and removed in *vacuo* using a rota vapor, resulting in viscous oils.

Reference and Control: *Ampicillin* was chosen as the reference for all bacteria species used: *E. coli*, *S. aureus* and *Klebsiella pneumoniae*. The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion²³⁻²⁴.

Antimicrobial tests: Plant extracts were investigated for their antimicrobial activity using the Disc Diffusion assay²³⁻²⁴.

Source of microorganisms: Gram negative (-) *E. coli*, *Klebsiella pneumoniae* and Gram positive (+) strains *Staphylococcus aureus* (ATCC 25923) were obtained from the Georgetown Public Hospital, GPH and stored in a refrigerator until required.

Positive control: In this study, tetracycline was used as a positive control to screen and analyze the antimicrobial properties of the different medicinal plants. This antimicrobial drug is clinically effective against both gram- negative as well as gram positive microbes. Side-effects from tetracycline are not common. Use of the antibiotic have known to cause stomach or bowel upsets and on rare occasions, allergic reactions.

In vitro Antimicrobial Susceptibility Tests: The Disc diffusion method was used to screen plant extracts for its *in vitro* antimicrobial activity. Plates were labeled according to extract, concentration and bacteria. Using the Disc diffusion assay²⁴, an inoculum containing bacteria cells were applied onto Mueller Hinton agar plates. A sterile swabbed was dipped into the bacteria culture and was uniformly spread on the surface of the Mueller Hinton agar. This was allowed to dry for 10 minutes. On each plate, four discs were placed equidistant using a sterilized tweesor. One of these is the reference disc onto which antibiotic was also applied and was used as the positive control: ampicillin for the bacteria. The reference antibiotic disc contained 200mg antibiotic/ml.

RESULTS:

TABLE 1: MEAN, STANDARD DEVIATION AND AREA OF ZONE OF INHIBITION FOR THE n-C₆H₁₄ AND CH₃CH₂OH EXTRACT OF SOLANUM MELONGENA AND MORINGA OLEIFERA

Sample	Pathogenic Microorganism	Concentration (%)	Mean Diameter	Mean Diameter with Standard deviation	Area of Zone of Inhibition (mm ²)
<i>Solanum melongena</i> Hexane	<i>E. coli</i>	5	4.43	4.43 ±3.85	15.04
		10	4.46	4.46 ± 2.97	15.65
		20	7.03	7.03 ± 0.25	38.79
	<i>S. aureus</i>	5	6.77	6.77 ± 1.04	35.87
		10	7.1	7.1 ± 0.22	39.57
		20	5.03	5.03 ± 2.53	19.86
	<i>Klebsiella spp</i>	5	2.33	2.33 ± 1.04	4.26
		10	7.97	7.97 ± 3.87	48.99
		20	7.17	7.17 ± 0.25	40.24
<i>Solanum melongena</i> Ethanol	<i>E. coli</i>	5	7.2	7.2 ± 0.71	40.69
		10	7.43	7.43 ± 0.30	43.33
		20	7.63	7.63 ±0.42	45.7
	<i>S. aureus</i>	5	7.87	7.87 ± 0.32	48.49
		10	7.73	7.73 ± 0.64	46.9
		20	8.27	8.27 ± 0.21	53.55
	<i>Klebsiella spp</i>	5	7.03	7.03 ± 0.11	38.79
		10	7.53	7.53 ± 0.32	44.51
		20	7.5	7.5 ±0.17	44.15
<i>Moringa oleifera</i> Hexane	<i>E. coli</i>	5	4.4	4.4 ±3.81	15.19
		10	7	7±0.2	38.46
		20	7.06	7.06 ±0.11	39.12
	<i>S. aureus</i>	5	4.66	4.66 ±4.07	17.04
		10	7.4	7.4 ±0.52	42.98
		20	7.53	7.53 ±0.49	44.51
	<i>klebsiella spp</i>	5	7.33	7.33 ± 0.28	42.17
		10	7.26	7.26 ± 0.20	41.37
		20	4.86	4.86 ± 4.23	18.54
<i>Moringa oleifera</i> Ethanol	<i>E. coli</i>	5	6.73	6.73 ±0.25	33.55
		10	4.76	4.76 ± 4.12	17.78
		20	7.73	7.73 ± 0.11	46.9
	<i>S. aureus</i>	5	5	5 ± 4.35	38.46
		10	8.1	8.1 ±0.79	51.5
		20	8.1	8.1±0	51.5
	<i>Klebsiella spp</i>	5	6.93	6.93 ±0.05	37.69
		10	7.33	7.33 ±0.05	42.17
		20	7.93	7.93 ±0.11	49.36

Positive control: (Table 2)**TABLE 2: AREA OF ZONE OF INHIBITION, ZOI FOR THE POSITIVE CONTROL, TETRACYCLINE AGAINST PATHOGENS**

Microorganism	Area of zone of inhibition (mm ²)
<i>Escherichia.coli</i>	36cm ²
<i>Staphylococcus. aureus</i>	37cm ²
<i>Klebsiella. pneumoniae</i>	35cm ²

DISCUSSION: Antimicrobial properties of *Solanum melogena* and *Moringa oleifera* C₂H₅OH and n-C₆H₁₄ extracts were investigated *in vitro* at concentrations of 5%, 10% and 20% using the Disc diffusion assay. Investigations were done against three pathogenic microorganisms: *E. coli*, *S. aureus* and *Klebsiella pneumoniae* using the Disc diffusion assay. The area of zone of inhibition was used as the gauge of the plant's antimicrobial properties. Larger the diameter of zone of inhibition, greater is the plant's antimicrobial activities. It is anticipated through the antimicrobial activity of plant extract, no area of growth will be induced around the disc. Bacteria colonies sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains grow up to the edge of the disc. Discs applied to the plates already streaked with bacteria and the fungus.

A comparison of the effect of the various solvent extracts against the three pathogenic microorganisms at three different concentrations can be discussed. In general, there seem to be an increase in the plant's extract antimicrobial activity as the concentration of the extract is increased. For example, *Solanum melongena* C₂H₅OH extract induces area of zone of inhibition (ZOI) of 40.69, 43.33 and 45.7 mm² against *E.coli* as the concentration of the plant extract increased from 5% to 20%. Likewise *Moringa oleifera* CH₃CH₂OH extract induces area of zone of inhibition of 37.69, 42.17 and 49.36 mm² against *Klebsiella pneumoniae* at concentration of 5, 10 and 20% of extract respectively.

However, there were exceptions to the above general increase in bacterial activity. For example, *Solanum melogena* n-C₆H₁₄ extract showed an increase in antimicrobial activity against *S.aureus* followed by a decrease at the 20% concentration. For example, area of zone of inhibition of 35.87 mm², 39.57 mm² and 19.86 mm² was observed at concentration of 5, 10 and 20% of extract. *Moringa oleifera* C₂H₅OH extract also showed a decreased in antimicrobial activity followed by an increase.

For example, against *Klebsiella* species value of 33.35 mm², 17.78 mm² and 46.0 mm² were obtained at the respective concentrations of 5, 10 and 20 % of extract. Of significance, there was a decrease in the area of zone of inhibition for *Moringa oleifera* hexane extract against *Klebsiella* species at all three concentrations. Area of zone of inhibition of 42.17 mm², 41.37 mm² and 18.54 mm² were obtained against *Klebsiella* species at concentrations of 5, 10 and 20% of extract. The highest area of zone of inhibition of 53.55 mm² induced by *Solanum melogena* C₂H₅OH extract against *S. aureus* at 20% concentration of extract.

The smallest area of zone of inhibition of 15.04 mm² was induced by *Solanum melogena* n-C₆H₁₄ extract against *E. coli*, where values of 15.04 mm², 15.65 mm² and 38.79 mm² were registered at the respective concentration. The C₂H₅OH extract of either plant seems to be more antimicrobial than the n-C₆H₁₄ extract, suggesting greater localisation of plant natural products antimicrobial agents or the interactions of natural products via non covalent interactions to produce novel antimicrobial systems or assemblies. For example, *Solanum melogena* n-C₆H₁₄ extract induces area of zone of inhibition of 35.37 mm², 39.57 mm² and 19.86 mm² against *S. aureus*. However, *Solanum melogena* CH₃CH₂OH extract induced area of zone of inhibition of 48.49 mm², 46.9 mm² and 53.53 mm² against *S. aureus* at concentration of 5%, 10% and 20% concentration respectively.

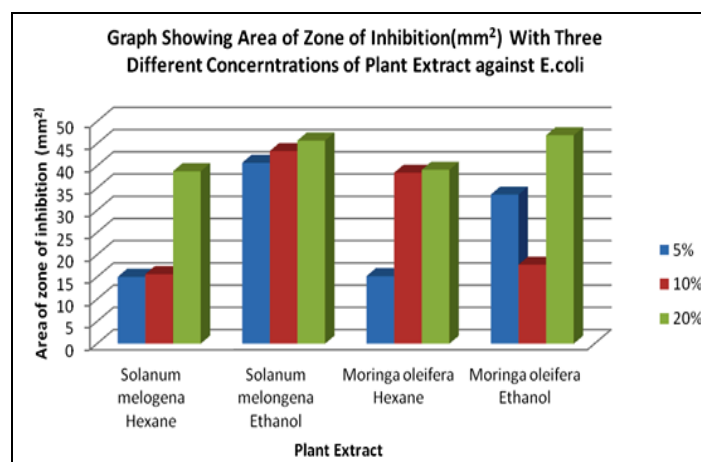


FIG. 2: AREA OF ZONE OF INHIBITION (mm²) OF PLANT EXTRACTS AGAINST E. COLI AT CONCENTRATION of 5, 10 and 20%

Graph 1 shows the area of ZOI (mm²) at 5%, 10%, & 20% concentrations of both plant extracts against colonies of *E. coli*.

From the graph it can be observed that the n-C₆H₁₄ extract of *Moringa oleifera* was more antibacterial. Values of 38.79 mm² and 39.12 mm² were recorded against *E. coli*. Also, at the 20% concentration, *Moringa oleifera* C₂H₅OH extract was more antimicrobial than *Solanum melongena*. Values of 46.9 mm² and 45.7 mm² were registered respectively.

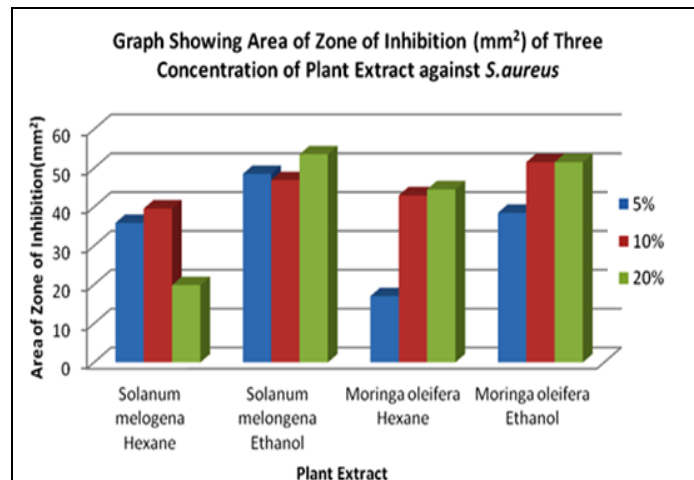


FIG. 3: AREA OF ZONE OF INHIBITION (mm²) OF PLANT EXTRACTS AGAINST *S. AUREUS* AT CONCENTRATION OF 5, 10 AND 20%.

Graph 2 shows the area of ZOI (mm²) at 5%, 10%, & 20% concentrations of both plant extracts against colonies of *S. aureus*. From the graph, the n-C₆H₁₄ extract of *Moringa oleifera* is more antimicrobial than that of *Solanum melongena*. Values of 44.51 mm² and 19.86 mm² were observed respectively. However, *Solanum Melongena* C₂H₅OH extract is more antimicrobial against *S. aureus* than *Moringa's* C₂H₅OH extract at the 20% concentration. Values of 53.55 mm² and 51.5 mm² were observed respectively.

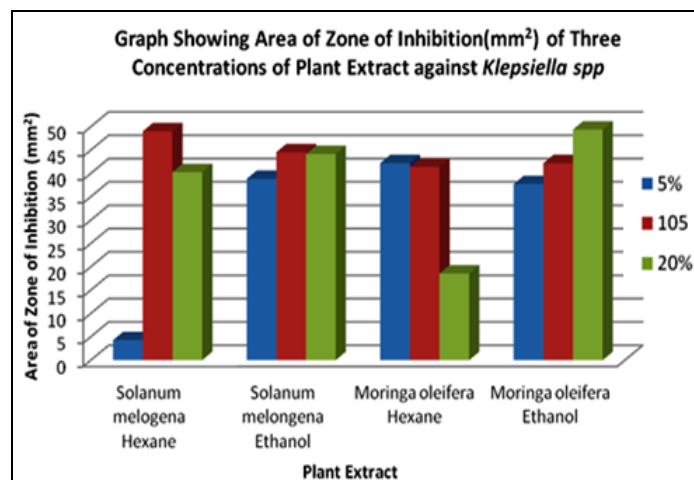


FIG. 4: AREA OF ZONE OF INHIBITION (mm²) OF PLANT EXTRACTS AGAINST *KLEPSIELLA SPECIES* AT CONCENTRATION OF 5, 10 AND 20%.

Graph 3 shows the area of ZOI (mm²) at 5%, 10% and 20% concentrations of plant extract against colonies of *Klebsiella pneumoniae*. From the graph it can be observed that the n-C₆H₁₄ extract of *Solanum melongena* induces a higher area of zone of inhibition against *Klebsiella pneumoniae* compared with *Moringa oleifera* at the 20% concentration. Values of 40.24 cm² and 18.54 cm² were registered respectively. Likewise, C₂H₅OH extract of *Solanum Melongena* were more antimicrobial than *Moringa oleifera* at 20% concentration of plant extract. Areas of ZOI registered were 44.15 mm² and 49.36 mm² respectively.

Antimicrobial activity was also investigated for the positive control, tetracycline against the pathogens. It is found that the diameter of the zone of inhibition, ZOI is less than that induced by the n-C₆H₁₄ and CH₃CH₂OH extract of both plants. This suggests and justifies the use of these fruits as potent antimicrobial agent in addition to their nutritional status.

CONCLUSION: From this study, it can be concluded that n-C₆H₁₄ and CH₃CH₂OH extract of *Solanum melongena* and *Moringa oleifera* possess antibacterial activity as significant zone of inhibition were observed. The area of ZOI ranges from 19.86 mm² to 53.55 mm². The CH₃CH₂OH extracts showed more potential antimicrobial properties than the n-C₆H₁₄ extract. The n-C₆H₁₄ and CH₃CH₂OH extract of both plants showed selective antimicrobial activity against the three pathogens: *E. coli*, *S. aureus* and *Klebsiella pneumoniae*. Against, *E. coli* and *S. aureus*, *Moringa oleifera* n-C₆H₁₄ is more resistant than *S. melongena*. *Solanum melongena* extract is more resistant against *Klebsiella pneumonia* compared to *Moringa oleifera* extract. For CH₃CH₂OH extract, against *E. coli* and *Klebsiella pneumoniae*, *Moringa oleifera* extract is more resistant. However, *Solanum melongena* extract is more resistant against *S. aureus*.

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