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**Original Article** 

#### Antibacterial potential of ethanol leave extracts of *Helianthus annuus*, *Moringa oleifera*, *Euphorbia heterophylla* and *Physalis angulata*

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#### Abstract

Natural substances with medicinal properties have been isolated from several plants and are advocated for use as antimicrobials. This study was aimed at evaluating the antibacterial activities of ethanolic leaf extracts of four plants against selected bacterial species. The plants examined were Helianthus annuus, Moringa oleifera, Euphorbia heterophylla and Physalis angulata while the bacterial species were Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Klebsiella sp. The minimum inhibitory concentration of the extracts was carried out, using the agar diffusion method while the growth profiles of the test bacterial species were carried out in broth media. The plant extracts were observed to contain phenol, terpenoids, saponins and tannins as phytochemical constituents. All the extracts showed inhibition against most of the test bacteria, although the degree of inhibition was observed to be directly proportional to the concentration used. However, *Klebsiella* sp was recorded to show resistance to most concentrations of the extracts with resistance to all concentrations of the Physialis angulata. The minimum inhibitory concentration (MIC) of all the extracts against Staphylococcus aureus and Bacillus subtilis was observed to be 500 mg/L. For Pseudomonas aeruginosa, MIC of 500 and 2000 mg/L were observed for *Physalis angulata* and *Euphorbia heterophylla*, respectively while 1000 mg/L was observed for the Helianthus annuus and Moringa oleifera. In presence of the extracts, extended lag period was observed during growth, when compared to the control broth culture. In some cases, growth was not evident in the broth cultures containing the extracts throughout the period of incubation. The findings of this study could help in the development of advanced products that can be of medical and pharmaceutical importance.

Keywords: Antibacterial, Growth inhibition, Plant extracts

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#### Introduction

Plant resulting diets have been used for their effective beneficial properties and have been in existence for several years for the treatment of diseases by traditional specialists. In Nigeria the use of herbal medicines is steadily growing, several substances from medicinal plants have biological activities (Subashini and Rakshitha, 2012; Falowo et al., 2014). The achievement level of current medication depends on the continuous search for natural drugs to overcome the encounters as a result of resistant strain bacteria challenges. In recent years many chemically synthesized drugs have been used in inhibition and treatment of various infectious diseases as this poses serious adverse effects on human and even on animals over time as they accumulate leading to organisms being resistant (Ughachukwu et al., 2014).

A number of plants are indicated to have both antibacterial and antioxidant constituents. These medicinal plants are recognized to have useful immune-modulatory action by instigating mutually non-specific and specific immunity (Ushimaru et al., 2007). For example, Helianthus annuus leaves are used as an infusion to manage high temperatures, lung complications and diarrhea (Alibe and Inuwa, 2012). The leaves contain several alkaloids, flavonoids, unstable oils and terpenoids necessary for numerous actions like antimicrobial activity, anti-tumor activity and antioxidant activity (Dwivedi and Sharma, 2014). Moringa oleifera is also generally well-known and used for its health benefits. It is being referred to as the miracle tree because of its remarkable medicinal properties for several diseases and even lasting infections. Some studies have been carried out to separate biologically active substances from several portions of the plant due to the numerous uses (Razis et al., 2014).

In addition, *Euphorbia heterophylla* is one of the numerous plants that possess therapeutic and pharmaceutical properties, which are used in all major systems of remedy for treating several infections. Presence of latex in the plant is one of the main reasons, it is considered to be a toxic plant. In spite of its toxicity properties, it is also known to possess numerous medicinal properties too. *Euphorbia heterophylla* is used broadly in African countries. Generally, this plant is regarded as a purgative, antiasthmatic, anti-inflammatory. It has also been reported to be oxytocic. This plant is used for the treatment of gonorrhea, lung infection, malaria, and

wart cure in traditional medicine. Furthermore, *Physalis angulata* is used regularly in the treatment of gonorrhea. Extracts of *Physalis angulata* have been indicated to possess antinociceptive, antibacterial and anti-inflammatory properties (Osho et al., 2010; Zubair et al., 2014).

It is therefore vital to produce some safer, more effective and also low-cost antimicrobial agents to fight these issues. The determination of a plant's antimicrobial profile against microorganisms may help for further tests towards its evaluation as a food preservative (Ahmadu and Omonigho, 2013). This study was therefore aimed at evaluating the antibacterial potentials of ethanol extracts of *Helianthus annus*, *Moringa oleifera*, *Euphorbia heterophylla* and *Physalis angulata* against selected bacterial pathogens.

#### **Material and Methods**

#### **Preparation of test isolates**

The bacterial isolates used for the study were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella* sp. Before use, the isolates were first streaked on nutrient agar plates to ascertain their purity before sub culturing in nutrient broth. Prior to use, a 24 h nutrient broth grown isolate was centrifuged and the cells suspended in normal saline (0.85 NaCl w/v). The suspended cells were stored at 4 °C, until when needed.

#### **Preparation of plant extracts**

The plants used for this study were Helianthus annuus, Moringa oleifera, Euphorbia heterophylla and Physalis angulata. The leaves of the plants were obtained from the environment of Landmark University, Omu Aran, Kwara State. The leaves of the plants were washed with clean water to remove all unwanted materials. The leaves were then air-dried and pulverized with a laboratory blender before soaking 200 g of the respective leaves in 500 mL of ethanol for extraction at 25 °C for 24 h (Sahraei et al., 2014). At the completion of the 24 h extraction period in the solvent, the extracts were filtered using filter paper, while the supernatants were discarded. The filtrates were then concentrated in a rotary evaporator and later freeze-dried, before storing in clean air-tight plastic bottles at 4 °C, until when required for use.

#### Preliminary assay of antibacterial potential

To evaluate the antibacterial potential of the extracts, 100 mL of sterile nutrient agar was left to cool to about

45 °C, after which 2 ml of normal saline suspended cells was added and swirled before dispensing in 20 ml quantity in petri dishes and allowed to solidify. The stock concentration of the crude extracts of all the plants was prepared in universal bottles. Using a cork borer, three wells were bored in each plate before adding 0.2 ml of the respective extracts in a well and allowed to diffuse. Following diffusion of the extract, the plates were incubated at 37 °C for 24 h. The zones of inhibition were observed and measured using a ruler and then recorded in millimeters appropriately. In each case, a control well that contained only the diluent used for preparation of the respective extracts was added. The diluent in this study was Tween 80.

#### Determination of minimum inhibitory concentration

To determine the minimum inhibitory concentration (MIC) of the extracts, different concentrations (500, 1000, 1500, 2000, 2500 and 3000 mg/L) were prepared. The agar well diffusion method was employed. Following incubation, the lowest concentration of the plant extracts at which the growth of the respective isolates is inhibited (identified by zone of inhibition) was indicated as the MIC. The MIC values were recorded for each extract against the test organisms.

## Determination of the phytochemical constituents of the plant extracts

The phytochemical constituents tested for were alkaloid, cardiac glycosides, terpenoid, steroids, flavonoids, tannins, saponins and phlobatannin. All phytochemical screenings were carried out using the protocol reported by Trease and Evans (2002) and Sofowora (1996).

#### Growth rate studies in broth

Growth rate studies were carried out in a liquid medium. To a 150 mL sterile nutrient broth, 3 mL of a known concentration of the respective extracts was added and incubated in an orbital shaker (S15200) at 37 °C at a shaking speed of 120 rpm. Immediately after inoculation and every 1 h interval for a 10 h duration, 5 mL of sample was withdrawn from each flask for measurement of absorbance at a wavelength of 720 nm, using a UV-VIS spectrophotometer.

Two sets of controls were setup. The first control was nutrient broth that was inoculated with a respective isolate but did not contain any extract while the second control was inoculated nutrient broth that contained known concentration of Tween 80 (Sahraei et al., 2014) Growth rate was calculated as:

Growth rate 
$$(d^{-1}) = \frac{\ln(C1) - \ln(C0)}{t1 - t0}$$

Where C0 and C1 represent initial and final absorbance, respectively

t0 and t1 represent initial and final time, respectively

All experimental setups were carried out in duplicates

#### Results

#### Phytochemical properties of the plant extracts

The phytochemical profile of the extracts revealed the presence of phenols, terpenoids, saponins and tannins in all the extracts. Steroids were not observed in any of the extracts except in *Helianthus annuus* (Table 1).

Phytochemical	Extracts			
	Helianthus Annuus		Euphorbia heterophylla	Physalis Angulata
Cardiac glycosides	-	+	-	+
Phenols	+	+	+	+
Anthraquinones	-	+	+	-
Terpenoids	+	+	+	+
Saponins	+	+	+	+
Alkaloids	-	+	+	-
Flavonoids	+	-	-	+
Tannins	+	+	+	+
Steroids	+	-	-	-
Phlobatannins	-	+	+	-

'+' and '- 'represent presence of phytochemical and absence of phytochemical, respectively.

## Growth inhibition of the *Staphylococcus aureus* in presence of the extracts

At the different concentrations of the Helianthus annuus extract minute or no growth of Staphylococcus aureus was observed in the culture broth, as against the control set up where growth became evident after 2 h of incubation. Generally extended lag periods of 6 h and 8 h were observed in the broths that contained extract concentrations of 1000 mg/L and 500 mg/L, respectively. In broth that contained the Moringa oleifera extract, no appreciable growth of the Staphylococcus aureus was observed until after 6 h of incubation. This trend was irrespective of the extract concentration in the broth (Fig. 1). In presence of the respective concentrations of the Euphorbia

*heterophylla* extract, growth of the *Staphylococcus aureus* was inhibited during the period of incubation. Similarly, extract of the *Physalis angulata* showed remarkable inhibition in growth of the *Staphylococcus aureus* in broth culture. This trend was also irrespective of the concentration of extract used (Fig. 1).

In nutrient agar plate, growth of the *Staphylococcus aureus* showed inhibition at the different concentrations of the extracts. This observation was irrespective of the extract used (Table 2).

## Table-2. Antibacterial activity of the extracts against *Staphylococcus aureus* in nutrient agar plates

Concentration mg/L	Helianthus annuus (mm)	Moringa oleifera (mm)	Euphorbia heterophylla (mm)	Physalis angulata (mm)
500	+(32)	+(18)	+(25)	+(45)
1000	+(16)	+(33)	+(26)	+(30)
1500	+(20)	+(15)	+(24)	+(31)
2000	+(15)	+16)	+(22)	+(20)
2500	+(17)	+(33)	+(17)	+(17)
3000	+(17)	+(17)	+(15)	+(15)

+ represents growth inhibition. Values in parenthesis indicate zones of inhibition

## Growth inhibition of the *Pseudomonas aeruginosa* in presence of the extracts

In the broth culture that contained the *Helianthus* annuus extract, growth of the *Pseudomonas* aeruginosa was inhibited until after 7 h, when growth was observed. This trend was irrespective of the different concentrations of the *Helianthus* annuus extract in the broth. In presence of the different concentrations of *Moringa* oleifera extract, *Pseudomonas* aeruginosa showed an extended lag phase of 6 h broth, when compared to the control setup that contained only extract where visible growth was observed from 2 h of incubation (Fig. 2).

At different concentrations of *Euphorbia heterophylla* extract, minute or no growth of *Pseudomonas aeruginosa* was observed in the culture broth containing the extract as against the control setup where growth was evident at 2 h of incubation. At the respective extract concentrations in the broth, only minute growth was observed throughout the 10 h incubation period. In presence of the *Physalis angulata* extract in the broth medium, *Pseudomonas aeruginosa* showed no appreciable growth until after 7 h incubation. This observation was irrespective of the extract concentration in the broth (Fig. 2).



Figure-1. Effect of the extracts on growth of Staphylococcus aureus



Figure-2. Effect of the extracts on growth of Pseudomonas aeruginosa

Generally, all the extracts showed inhibition of the *Pseudomonas aeruginosa* in agar plates. However, none of the extracts (with the exception of *Physalis* angulata) showed inhibition when used at concentration of 500 mg/L (Table 3).

 Table 3: Antibacterial activity of the extracts against *Pseudomonas aeruginosa* in nutrient agar plates

Concentration mg/L	Helianthus annuus (mm)	Moringa oleifera (mm)	Euphorbia heterophylla (mm)	Physalis angulata (mm)
500	-	-	-	+(29)
1000	+(23)	+(15)	-	+(25)
1500	+(13)	+(13)	-	+(25)
2000	+(20)	+(24)	+(13)	+(20)
2500	+(18)	+(28)	+(11)	+(24)
3000	+(20)	+(14)	+(12)	+(22)

<sup>&#</sup>x27;+' and '- 'represent growth inhibition and no growth inhibition, respectively. Values in parenthesis indicate zones of inhibition

## Growth inhibition of the *Bacillus subtilis* in presence of the extracts

At different concentrations of Helianthus annuus extract, minute or no growth of the Bacillus subtilis was observed in the culture broth against the control set up where growth was evident from 2 h of incubation. Generally extended lag period of 8 h was observed in broth with extract concentration of 500 mg/L, Bacillus subtilis showed no considerable growth in extract of concentration 1500mg/L. Bacillus subtilis grown on Moringa oleifera extract showed no appreciable growth until the 6 h when the organism considerable growth recorded on different concentration of extract, compared to the control set up which showed visible growth (Fig. 3).

At different concentrations of the *Euphorbia heterophylla* extract appreciable growth of *Bacillus subtilis* was observed in the culture broth containing the extract in 500, 1000 and 2000mg/L against the control set up where growth was evident at 2 h of incubation, there was no appreciable growth of organism was observed in 1500mg/L of the extract in the culture broth. *Bacillus subtilis* showed no considerable growth in medium containing *Physalis* 

*angulata* extract of concentration 1000 and 1500mg/L (Fig. 3).

In nutrient agar plates, all the extracts inhibited the growth of the *Bacillus subtilis*. This was irrespective of the concentration of the extract used (Table 4).

 Table-4. Antibacterial activity of the extracts against *Bacillus subtilis* in nutrient agar plates

Concentration (mg/L)	Helianthus annuus (mm)	Moringa oleifera (mm)	Euphorbia heterophylla (mm)	Physalis angulata (mm)
500	+(13)	+(20)	+(20)	+(17)
1000	+(16)	+(17)	+(30)	+(15)
1500	+(18)	+(20)	+(25)	+(20)
2000	+(15)	+(17)	+(20)	+(40)
2500	+(14)	+(30)	+(20)	+(35)
3000	+(14)	+(18)	+(15)	+(45)

'+' represents growth inhibition. Values in parenthesis indicate zones of inhibition

## Growth inhibition of the *Escherichia coli* in presence of the extracts

*Escherichia coli* grown on *Helianthus annuus* extract shows a lag period throughout the study in 500, 1000 and 2000mg/L. There was an appreciable growth of organism seen culture broth containing extract in 1500mg/L as against the control setup. In the nutrient broth that contained the *Moringa oleifera* extract, no appreciable growth of *Escherichia coli* was observed until after 5 h of incubation. No appreciable growth was observed in medium with extract concentration of 1500 mg/L. Minute growth of the organism was observed in 500, 1000, 2000mg/L of the extract compared with the control which showed appreciable growth from 2 h of incubation (Fig. 4).

At different concentrations of *Euphorbia heterophylla* extract, minute or no growth of *Escherichia coli* was observed in the culture broth against the control set up where growth was evident. A similar observation was observed in presence of the *Physalis angulata* extract. And this was irrespective of the extract concentration in the broth medium (Fig. 4).



Figure. 3: Effect of the extracts on growth of Bacillus subtilis



Figue.4: Effect of the extracts on growth of Escherichia coli

In agar plates, the different concentrations of the *Helianthus annus, Moringa oleifera* and *Physalis angulata* extracts were found to inhibit the growth of *Escherichia coli* while 500 mg/L concentration of the *Euphorbia heterophylla* extract did not show any inhibition to growth of the *Escherichia coli* (Table 5).

 Table-5.
 Antibacterial activity of the extracts against *Escherichia coli* in nutrient agar plates

Concentration (mg/L)	Helianthus annuus (mm)	Moringa oleifera (mm)	Euphorbia heterophylla (mm)	Physalis angulata (mm)
500	+(25)	+(10)	-	+(32)
1000	+(30)	+(25)	+(34)	+(34)
1500	+(28)	+(33)	+(21)	-
2000	+(25)	-	+(24)	+(32)
2500	+(25)	+(30)	+(12)	+(30)
3000	+(25)	+(25)	+(37)	+(30)

'+' and '-'represent growth inhibition and no growth inhibition respectively. Values in parenthesis indicate zones of inhibition

### Growth inhibition on the *Klebsiella* sp in presence of the extracts

At different concentrations of *Helianthus annus* extract, there was an appreciable growth of the

organism alongside the control setup which showed visible growth 2 h of incubation. Similarly, *Klebsiella* sp grown on the *Moringa oleifera* extract showed a visible growth at different concentrations in the broth medium (Fig. 5). In medium that contained the *Euphorbia heterophylla* extract notable growth of the *Klebsiella* sp was observed at the different concentrations. As was observed in presence of the other extracts, no inhibition of the *Klebsiella* sp was observed in broth medium containing the *Physalis angulata* extract (Fig. 5).

 Table-6.
 Antibacterial activity of the extracts against *Klebsiella* sp in nutrient agar plates

Concentration (mg/L)	Helianthus annuus (mm)	Moringa oleifera (mm)	Euphorbia heterophylla (mm)	Physalis angulata (mm)
500	-	-	-	-
1000	-	-	-	-
1500	-	-	-	-
2000	+(25)	-	+(35)	-
2500	+(30)	+(25)	+(35)	-
3000	+(25)	-	+(30)	-

'+' and '- 'represent growth inhibition and no growth inhibition, respectively. Values in parenthesis indicate zones of inhibition.



Figure-5. Effect of the extracts on growth of Klebsiella

Generally, in nutrient agar plates, none of the extracts inhibited growth of the *Klebsiella* sp at concentrations less than 2000 mg/L (Table 6).

#### Discussion

The test bacteria used for this study were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella* sp. The isolates were selected because they are common pathogens in human and animal infections. Besides, majority of these organisms are indicated to be highly pathogenic and their rate of infection has increased considerably in recent years (Islam et al., 2016).

The antimicrobial potential of *Helianthus annuus* has been investigated against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*, reported by earlier workers (Al-Snafi, 2018). In the study, the oil was observed to possess inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*. Similarly, the ethanol extract of the stem is indicated to have inhibitory activity against *Staphylococcus aureus*, *Eschericha*  *coli, Aspergillus niger* and *Candida albicans* (Alibe and Inuwa, 2012). In addition, the ethanol extract has been indicated to be effective in inhibiting the growth of *Staphylococcus aureus, Aspergillus niger* and *Candida albicans*, with *Escherichia coli* reported to be resistant to its inhibitory activity (Subashini *and* Rakshitha, 2012; Adetunji et al., 2014).

Also, the antimicrobial activity of the aqueous and ethanol extracts of the leaves showed inhibition against Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, **Bacillus** subtilis. Escherichia coli, Salmonella typhimurium and Micrococcus luteus (Ibrahim et al., 2014). The methanol extract of the seeds has also been reported to show antimicrobial activity against Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Vibrio cholera, Rhizopus stolonifer, Aspergillus fumigatus, Fuserium oxisporium and Candida albicans, with high, moderate and less activities reported against Salmonella typhi, Staphylococcus aureus and Vibrio cholera, respectively (Adetunji et al., 2014). In another study, the methanol, ethyl acetate and petroleum ether of Helianthus annuus was reported to show high inhibitory activity against Salmonella typhi,

moderate inhibition against *Pseudomonas aeruginosa* and less sensitivity to *Vibrio cholerae* (Islam et al., 2016).

All the concentrations of the Moringa oleifera used in this study showed inhibition against the growth of the test bacterial species except for Klebsiella sp. where inhibition was only observed at high concentrations of the extract. In the findings reported by Singh and Tafida, (2013), the aqueous, ethanol and methanol extracts of the leaves have been reported to have Escherichia inhibitory ability against coli. Staphylococcus aureus and Pseudomonas aeruginosa. The study further revealed that the inhibitory effects of the extracts were significantly higher on **Staphylococcus** Escherichia coli than on aureus and Pseudomonas aeruginosa (Singh and Tafida, 2013).

In another study on the antimicrobial action of the leaf extracts of Moringa oleifera against selected bacteria, the findings revealed significant antimicrobial activity of the extract against Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Sarcina lutea, Escherichia coli and Mycobacterium phlei (Pal et al., 1995). Also, when the antibacterial activity potential of aqueous, ethanol, petroleum ether and chloroform extracts were investigated in another study, the findings revealed maximum inhibition against the growth of Staphylococcus aureus (Kalpana et al., 2013). Furthermore, in a study on the antimicrobial activities and phytochemical analysis of petroleum extract of Moringa oleifera leaves on Staphylococcus aureus and Streptococcus species, it was indicated that significantly more activity was observed against the growth of Streptococcus species than Staphylococcus aureus (Ajayi and Fadeyi, 2015).

In this study, *Euphorbia heterophylla* exhibited inhibition against the growth of the test bacteria, with a minimum inhibitory concentration (MIC) of 500mg/L, except for *Klebsiella* sp and *Pseudomonas aeruginosa* where inhibition was only observed at high concentrations of the extract. A high antibacterial activity of *Euphorbia heterophylla* against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* has been reported by earlier investigators (Ughachukwu et al., 2014). The study observed that growth inhibition in presence of the extract was reliant on the dose of extract used. A minimum inhibitory concentration of 6.25 mg/mL of the extract was reported for *Staphylococcus aureus* and *Pseudomonas aeruginosa* while MIC of 12.5 mg/L was reported for *Klebsiella* sp and *Escherichia* coli (Ughachukwu et al., 2014).

The methanol and aqueous leaf extracts of Euphorbia heterophylla have also been shown to have antibacterial potential on Salmonella typhi, Shigella flexneri, Escherichia coli, and Proteus vulgaris. The extracts were observed to exhibit antibacterial activity against the test organisms at concentrations of 150 mg/mL and 200 mg/mL, for the methanol and aqueous extracts, respectively (Oyedum, 2017). Although the Physalis angulata showed inhibition against most of the test bacterial species, none of the concentrations used inhibited the growth of the Klebsiella sp. Growth inhibition was however observed to be dependent on the concentration of extract used. A similar observation has been reported by (Zubair et al., 2014). In their report, the zones of inhibition observed in presence of the ethanolic extract of Physalis angulata ranged from 8.2 mm to 17.2 mm and were dependent on the concentration (Zubair et al., 2014). In another study, the antimicrobial action of essential oil extracted from aerial parts of Physalis angulata against selected bacteria, revealed sensitivity to Bacillus subtilis and Klebsiella sp, with MIC of 4.0 mg/mL. However, the extract was not observed to inhibit the growth of Pseudomonas aeruginosa and Staphylococcus aureus (Osho et al., 2010).

The plant extracts were also seen to possess some phytochemical constituents such as phenol, terpenoids, saponins and tannins were present in all the extracts. In a similar study by Eze et al., (2015) it was Helianthus shown that annuus contained phytochemicals mainly tannins and flavonoids which are recognized to bring about antibacterial actions because of the control of capability to deactivate microbial bonds, microbial enzymes and microbial cell envelope. In the report by Zubair et al., (2014) the test of the presence of phytochemicals in the ethanol extract of *Physalis angulata* plant showed an extensive group of phytochemical constituent, alkaloids, tannins, saponins, steroids, terpenoids, phenols, flavonoids were present except saponins.

#### Conclusion

The study revealed that all the test leave extracts (*Helianthus annuus*, *Moringa oleifera*, *Euphorbia heterophylla*, *Physalis angulata*) showed inhibition against the growth of the isolates in solid media. In addition, extended lag periods were observed for the isolates in liquid cultures that contained the extracts.

Also observed in this study was the resistance of *Klebsiella* sp to all concentrations of *Physalis* angulata.

Although there is the need to isolate the active ingredients in these crude extracts for further studies, findings from this study could help in the development of advanced products that could be of medical and pharmaceutical importance.

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#### **Contribution of Authors**

Akpor OB: Conceived idea, designed research methodology, data collection, literature review, data analysis, manuscript writing, manuscript final reading and approval.

Hussein FU: Data collection, literature review, data analysis, manuscript writing, manuscript final reading and approval.

Oluba OM: Data collection, literature review, data analysis, manuscript writing, manuscript final reading and approval.

Olaolu TD: Designed research methodology, data collection, literature review, data analysis, manuscript writing, manuscript final reading and approval. Alabi OO: Literature review, data analysis, manuscript writing, manuscript final reading and approval. Shoyombo AJ: Literature review, data analysis, manuscript writing, manuscript final reading and approval.