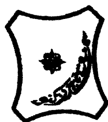
*Special Conference Edition, November, 2019*
**http://dx.doi.org/10.4314/bajopas.v12i1.6S**



***Bayero Journal of Pure and Applied Sciences, 12(1): 31 - 38***
**ISSN 2006 – 6996**

# COMBINED ACTIVITY OF *Azadirachta indica* and *Dodonea viscosa* LEAVES EXTRACT ON SOME SELECTED PATHOGENS

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***ABSTRACT***
***The study was undertaken to investigate the antimicrobial activity both individually and in combination of two Nigerian plants (Azadirachta indica and Dodonea viscosa) against a variety of pathogenic bacteria. Five bacterial species namely; Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae and Pseudomonas aeruginosa were selected for the assays. The powdered plant materials of the two plants were extracted using sohxlet extraction technique with methanol water and petroleum ether as solvents. The crude extracts of the two plants were subjected to phytochemical screening for qualitative detection of plant secondary metabolites. The extracts were further tested for antibacterial activity against the selected pathogens singly and then combined using agar well diffusion method. Antibacterial activity of the two plants singly indicates that the methanolic extract possess the highest antibacterial activity at a concentration of 50mg/ml with an inhibition zone of 23.3±0.5mm compared to aqueous extract with 21.7±0.5mm inhibition zone at a concentration of 50mg/ml. The least activity was observed with the petroleum ether extract with an inhibition zone of 7.3±0.5mm at a concentration of 12.5mg/ml. Combination of the plants extracts exhibited lower antibacterial activity on the test isolates compared to single plants as evidenced by the production of lower inhibition zones.***
***Key words: Azadirachta indica, Dodonea viscosa, pathogens, antimicrobial activity, synergistic effect***

## INTRODUCTION

The use of medicinal plants for treatment of various infections in traditional communities has been an age-long global practice. It has been estimated that 80% of African population use herbal regimen for treatment and control of diseases (Hugo and Russell, 2016). This provides a rationalization for studying medicinal plant extracts as a possible source of alternative therapy against infections. The primary benefit of using herbal drugs is that they are relatively safer and cheaper than the synthetic alternatives (Aiyegoro and Okoh, 2009).

Presently, the trend of events points towards utilizing everything "natural" including medicine and in this regards medicinal plants are value added for the content and chemical composition of their active components. In a wider context, there is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc (Vennaposa *et al.,* 2013).

Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases among other factors. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors (Abiramasundari *et al.,* 2011). Thus, this situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, coupled with cost production of synthetic drugs which is high in addition to them producing adverse effects compared to plant derived drugs.

*Azadirachta indica* (neem) is an evergreen and fast growing tree in the mahogany family Meliaceae; native to the Southeast Asia and found in most tropical countries. It is popularly known as neem tree in English and darbejiya, maina or dogon yaro (tall boy) in (Hausa) in Nigeria. *Azadirachta indica* is used to treat gastrointestinal upset, diarrhoea and intestinal infections, skin ulcers and infections and malaria (Anyaehie, 2009). The hypoglycaemic action of its leaves, stem and bark and seed oils as well as other medicinal uses of it and pharmacologically reported biological actions have been articulated in a review by Biswas *et al*. (2002)

*Dodonea viscosa* is a shrub of flowering plant in the soapberry family, Sapindaceae. The centre of origin is believed to be Australia, but occurs throughout the tropics and subtropics, Africa, Mexico, New Zealand, India, Northern Mariana Island, Florida, Arizona, South America and elsewhere (Sandhya *et al*., 2009). Commonly called Pribet or Fir-Fir among the Hausa speaking people of Northern Nigeria, It has many medicinal properties and has been used by native people from all regions where it is found. It is a traditional medicine worldwide, administered orally or as a poultice to treat a great variety of ailments (Chong, 2003; Aliyu 2006). Digestive system disorders, including indigestion, diarrhoea and constipation are commonly treated in traditional medicine with an orally administered decoction of either leaves or roots (Sandhya *et al.,* 2009). Trachoma is treated with applications of leaf juice, and powdered leaves are given to expel roundworms (Sandhya *et al.,* 2009).

The present study seeks to study the *in vitro* synergistic antibacterial activity of *Azadirachta indica* and *Dodonea viscosa* against S*. aureus, E.coli, S. typhi, K. Pneumoniae and P. aeruginosa.*

## MATERIALS AND METHODS

### Collection and identification of plant materials

Matured leaves of *Azadirachta indica* and *Dodonea viscosa* were collected by hand plucking from parent plants spotted at different locations at Bayero University Kano new and old campus. All the plant materials were identified according to Demetrio *et al*. (2015) at the Department of Plant Biology Bayero University Kano. Herbarium accession number was given to each of the plants used and *Azadirachta indica* was identified as BUKHAN 312 and *Dodonea viscosa* as BUKHAN 0118.

### Drying and powdering of plant material

The plant materials were washed thoroughly several times with running tap water and kept under shade at room temperature for drying. After drying, the plant materials were grinded into powder and kept in a sealed plastic bag duly labelled individually as described by Mukhtar and Tukur (1999).

### Extraction procedure

The extraction procedure was carried out according to the methods described by Fatope and Hamisu (1999). One thousand (1000) ml of solvent (methanol, pet ether and distilled water) was added into a round bottom flask onto which thimble containing hundred grams (100g) of powdered plant using sohxlet extractor. All extracts were kept at $4^{o}$C until further use.

### Preparation of various concentration of the extract

This was carried out according to the method described by Taura and Oyeyi, (2009). One gram (1000mg) of each plant extract was reconstituted individually in 10ml dimethyl sulphoxide (DMSO) for methanol and petroleum ether extract and distilled water for aqueous extract to obtain a 100mg/ml solution. The 100mg/ml solution was then diluted with an equal volume of distilled water (100mls) for aqueous extract and DMSO for both methanolic and petroleum ether extracts to obtain a 50mg/ml solution. Serial double dilution procedure was continued to obtain lower concentration of the extracts (25mg/ml, 12.5mg/ml and 6.25mg/ml respectively.

### Test organisms and Biochemical test

The test organisms were pathogenic strains of *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* obtained from microbiology Department of Aminu Kano Teaching Hospital (AKTH) Kano, Nigeria. The test bacterial identity was characterised by observing their cultural growth characteristic and various biochemical tests as described by Cheesbrough, (2006).

### Standardisation of inoculums

The isolates were subcultured onto sterile nutrient agar plates incubated at $37^{o}$**C** for 24 hours. Using a sterilized wire loop, the overnight cultures were diluted in normal saline (0.85% w/v) such that their turbidity matches with 0.5 Macfarland standards which give a mean of $1.0 \times 10^{8}$ cfu/ml microbial population density (Cheesbrough, 2000).

**Phytochemical screening of the extracts**

All the plants extracts were subjected to phytochemical analysis to determine some of its secondary metabolites such as saponins, tannins, alkaloids, terpenoid, flavonoid, phenol and cardiac glycosides in the plants extract. The presence of alkaloids was detected with Dragendorff's reagent (Firdouse and Alam 2011) and Wagner's reagent (Lalitha and Jayanthi 2012), whereas Flavonoids by sodium hydroxide test and aluminum chloride test (Subash *et al*. 2013), and phenol with phoshomolybdic acid reagent (Kumar *et al*. 2007). Terpenoids was identified with chloroform and sulphuric acid (Edeoga *et al*. 2005) and saponins by froth test (Parekh and Chanda 2007). Tannins were spotted with ferric chloride test (Kumar *et al*., 2007; Parekh and Chanda 2007) and lead acetate test whereas anthraquinones by Borntrager's test and cardiac glycosides by Keller-Kiliani test and Legal test (Singh, 2012; De *et al*., 2010).

**Bioassay for antibacterial activity**

This was carried out according to the method described by Irshad *et al*. (2011). An inoculum suspension was swabbed uniformly on Mueller-Hinton Agar (MHA) and was allowed to dry for 5 minutes. Six (6) holes of 6 mm in diameter were made in the seeded agar using 6mm cork borer and 0.1ml of the four different concentrations of the plants extracts was added into separate well on the seeded medium. The plate were allowed to stand on the bench for 1 hour for proper diffusion and thereafter incubated at 37$^{o}$C for 24 hours. Antibacterial activity of each extract was expressed in terms of the diameter of zone of growth inhibition in millimeters (mm).

**Combined effects between the two plant extracts**

Agar well diffusion method was used. To identify the synergistic effect between the plants extract, the crude leaf extracts was mixed in equal (1:1) and different ratio in combination of two extracts (2:1 and 1:2). The plates were then incubated at 37$^{o}$C for 24 hours and the diameter of clearing zone was measured in millimeters (Thakur *et al*., 2012).

**Statistical analysis**

Data generated from the study was subjected to analysis of variance (ANOVA) and a p-value of 0.05 or less ($p<0.05$) was considered significant at 95% confidence interval.

**RESULTS**

The result in Table 1 revealed that the methanolic leaf extract (AME) of *A.indica* had the highest percentage yield of 32% followed by *A.indica* leaf aqueous extract (23.4%) and *A. indica* leaf petroleum ether extract (12%). The methanolic leaf extract of *D. viscosa* also has the highest percentage yield of 39% compared to the aqueous extract (25%) and petroleum ether extract (14%). Data from Table 2 show that phytochemical components including saponins and phenols were shown to be present in all the extract. Tannin was present in all the extract but was absent in (methanolic and petroleum ether) leaves extract of *A.indica*. Flavonoid was only present in *A. indica* methanolic and *D. viscosa* (methanolic and petroleum ether) leaves extract. Volatile oil was present in all the extracts except in *A.indica* methanolic extract. Glycoside was present only in *A. indica* aqueous extract and methanolic extract of *D.viscosa.* Steroids was present in the (methanolic and petroleum ether extract) of *A. indica* and *D. viscosa* (aqueous and petroleum ether extract). Terpenoids were present in all the extract except in *A.indica* (methanolic and petroleum ether) and *D. viscosa* (petroleum ether extract).

Table 1: **Physical characteristics of *Azadirachta indica* and *Dodonea viscosa* leave extract**

| Plants extract | % yield | Colour | Boiling point | Texture |
|---|---|---|---|---|
| AME | 32 | Dark green | 65$^{o}$C | Sticky |
| AAE | 23.4 | Dark brown | 100$^{o}$C | Gummy |
| APE | 12 | Dark green | 40$^{o}$C | Oily/Sticky |
| DME | 39 | Dark green | 65$^{o}$C | Sticky |
| DAE | 25 | Dark brown | 100$^{o}$C | Gummy |
| DPE | 14 | Dark green | 40$^{o}$C | Oily/sticky |

**AME** = *Azadirachta indica* leaves methanolic extract, **AAE**= *Azadirachta indica* leaves aqueous extract, **APE**= *Azadirachta indica* leaves petroleum ether extract, **DME**= *Dodonea viscosa* leaves methanolic extract, **DAE**= *Dodonea viscosa* leaves aqueous extract**,** **DPE**= *Dodonea viscosa* leaves petroleum ether extract

Table 2: **Phytochemical constituents of *Azadirachta indica* and *Dodonea viscosa* leaves extract**

| Phytochemical components | AME | AAE | APE | DME | DAE | DPE |
|---|---|---|---|---|---|---|
| Alkaloids | - | - | + | - | + | + |
| Tannins | - | + | - | + | + | + |
| Saponins | + | + | + | + | + | + |
| Flavonoids | + | - | - | + | - | + |
| Volatile oils | - | + | + | + | + | + |
| Glycosides | - | + | - | + | - | - |
| Steroids | + | - | + | - | + | + |
| Terpenoids | - | + | - | + | + | - |
| Phenols | + | + | + | + | + | + |

Keys **: AME**= *Azadirachta indica* leaves methanolic extract, **AAE**= *Azadirachta indica* leaves aqueous extract, **APE**= *Azadirachta indica* leaves petroleum ether extract; **DME**= *Dodonea viscosa* leaves methanolic extract, **DAE** *= Dodonea viscosa* leaves aqueous extract; **DPE**= *Dodonea viscosa* leaves petroleum ether extract,

Table 3 showed that *Azadirachta indica* leaves extract exhibited an antibacterial activity against all the bacteria tested with the exception of *Escherichia coli* and *Pseudomonas aeruginosa* which were resistant to the methanolic and aqueous extract although the petroleum ether extract exhibited some activity against all the tested isolates, the zone of inhibition was lower compared to the methanolic extract. The methanolic extract produces inhibition zones that range from 14.7±0.5mm to 23.3±0.5mm against the test organisms, whereas the aqueous and petroleum ether extract produces an inhibition zone that ranges from 13.3±0.5mm to 21.00±1.00mm and 8.3±0.5mm to 21.00±1.0mm against the test organism at various concentrations.

**Table 3: Antibacterial activity of *Azadirachta indica* leaves extract on the test isolates**

| | Zone of inhibition(mm) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Organism** | **AME (mg/ml)** | | | | **AAE (mg/ml)** | | | | **APE (mg/ml)** | | | | **Control** |
| | **50** | **25** | **12.5** | **6.25** | **50** | **25** | **12.5** | **6.25** | **50** | **25** | **12.5** | **6.25** | **25** |
| *S. aureus* | 23.3±0.5 | 20.7±1.00 | 19.0±1.0 | 17.3±0.5 | 19.0±0.0 | 17.0±1.0 | 16.3±0.5 | 13.3±0.5 | 21.0±1.0 | 19.3±0.5 | 17.3±0.5 | 15.3±0.5 | 27.0±1.0 |
| *E. coli* | 0.0±0.0 | 0.0±0.00 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 15.0±0.0 | 14.0±0.0 | 10.0±1.0 | 9.0±1.00 | 34.0±0.0 |
| *S. typhi* | 18.0±1.0 | 15.0±1.00 | 14.7±0.5 | 13.3±0.5 | 20.3±0.5 | 16.3±0.5 | 15.0±1.0 | 12.3±0.5 | 19.3±0.5 | 17..3±0.5 | 15.0±0.0 | 13.0±1.0 | 32.0±0.0 |
| *K.pneumoniae* | 16.0±0.5 | 16.3±0.5 | 15.0±0.5 | 10.3±0.5 | 21.7±0.5 | 20.0±1.0 | 15.0±0.5 | 8.3±0.5 | 11.0±1.0 | 10.3±0.5 | 9.0±0.0 | 8.0±0.00 | 25.0±1.0 |
| *P. aeruginosa* | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.00 | 15.7±0.5 | 9.0±0.00 | 9.0±1.0 | 8.3±0.5 | 23.0±0.0 |

Key: **AME**: *Azadirachta indica methanolic* extract, **AAE**: *Azadirachta indica* aqueous extract, **APE**: *Azadirachta indica* petroleum ether extract

Table 4 shows the inhibitory antibacterial activity of *D. viscosa* leaves against the tested isolates. The methanolic extract has the highest activity followed by the aqueous extract while the petroleum ether extract produced the lowest activity. The methanolic extract produces inhibition zone that ranges from 9.0±0.00mm to 13.3±0.5mm against the test organism, whereas the aqueous and petroleum ether extract ranges from 7.3±0.5mm to 11.0±1.00mm and 7.3±0.5mm to 9.7±0.5mm against the test organism at various concentration. *Escherichia coli* and *Pseudomonas aeruginosa* resisted the petroleum ether extract at various concentration used. The result of the study further indicates that the methanolic extract of *D.viscosa* produced a higher inhibition zone against *Salmonella typhi* than the other test organisms with a zone of inhibition of upto 13.3±0.5mm at a concentration of 50mg/ml compared to 7.0±0.00mm for *Escherichia coli* at the same concentration.

**Table 4: Antibacterial actvity of *Dodonea viscosa* leaves extract on the test isolates**

| Organism | Zone of inhibition(mm) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DME(mg/ml) | | | | DAE(mg/ml) | | | | DPE(mg/ml) | | | | Control |
| | 50 | 25 | 12.5 | 6.25 | 50 | 25 | 12.5 | 6.25 | 50 | 25 | 12.5 | 6.25 | 25 |
| *S. aureus* | 10.3±0.5 | 9.0±0.0 | 8.3±0.5 | 0.0±0.0 | 8.3±0.5 | 8.7±1.0 | 7.7±1.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 27±1.0 |
| *E. coli* | 7.0±0.0 | 7.3±0.5 | 7.7±0.5 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 34.0±0.0 |
| *S. typhi* | 13.3±0.5 | 12.0±0.0 | 10.3±0.0 | 9.0±0.0 | 8.3±0.5 | 8.3±0.5 | 7.7±1.0 | 7.3±0.5 | 9.7±0.5 | 7.7±0.5 | 7.3±0.5 | 0.0±0.0 | 32.0±0.0 |
| *K.pneumoniae* | 9.0±0.0 | 9.7±0.5 | 0.0±0.0 | 0.0±0.0 | 11.0±1.0 | 10.3±1.0 | 10.0±1.0 | 9.0±1.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 25.0±1.0 |
| *P. aeruginosa* | 10.0±0.0 | 7.7±0.5 | 0.0±0.0 | 0.0±0.0 | 9.3±0.5 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 23.0±0.0 |

**Keys**: **DME**: *Dodonea viscosa* methanolic extract, **DAE**: *Dodonea viscosa* aqueous extract, **DPE**: *Dodonea viscosa* petroleum ether extract

In Table 5, the combined activity was achieved by combining the different extracts at a ratio of 1:1, 2:1 and 1:2 at 50mg/ml concentration. The combinations are as follows; *A. indica* methanolic plus *D. viscosa* methanolic, *A. indica* aqueous plus *D. viscosa* aqueous, and *A. indica* petroleum ether plus *D.viscosa* petroleum ether. Various concentrations of these combinations were tested against the test bacteria. Compared with the activity of the plants singly, the combined plant extract exhibited lower inhibition zones against the bacterial isolates tested. Thus, no synergism was observed at all the ratios used. The methanolic extract of the two plants when combined produced a zone of inhibition of 13.±0.5mm at 1:1 ratio against *Staphylococcus aureus* compared to the individual extract of *A.indica* with the highest zone of 23.3±0.5mm against same isolate. Thus, synergistically the combined plant extracts had lower antibacterial activity.

Similarly, the aqueous and petroleum ether extract also show similar activity. Some extract that had antibacterial activity individually on the test isolate were unable to inhibit the growth of the organisms when combined.

**Table 5: Combined antibacterial activity of *A. indica* methanolic, aqueous and petroleum ether extract with *D. viscosa* methanolic, aqueous and petroleum ether extract on the test isolates**

| | AME/DME(50mg/ml) | | | AAE/DAE(50mg/ml) | | | APE/DPE(50 mg/ml) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ratio | | | Ratio | | | Ratio | | |
| Organisms | 1:1 | 2:1 | 1:2 | 1:1 | 2:1 | 1:2 | 1.1 | 2:1 | 1:2 |
| *S. aureus* | 13 | 11 | 9 | 0 | 12 | 10 | 12 | 0 | 0 |
| *E. coli* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *S. typhi* | 10 | 8 | 7 | 0 | 8 | 13 | 0 | 0 | 0 |
| *K. pneumonia* | 12 | 0 | 0 | 13 | 0 | 9 | 0 | 0 | 8 |
| *P. aeruginosa* | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**KEY: AME**: *Azadirachta indica* methanolic extract, **AAE:** *Azadirachta indica* aqueous extract **APE**: *Azadirachta indica* petroleum ether extract **DME**: *Dodonea viscosa* methanolic extract **DAE**: *Dodonea viscosa* aqueous extract **DPE:** *Dodonea viscosa* petroleum ether extract

**DISCUSSION**

The findings from this study demonstrates that the phytochemical analysis conducted on *A. indica* leaves extracts revealed the presence of alkaloids, tannins, flavonoids, steroids, saponins, phenols, cardiac glycosides which corresponds with the report of Aslam *et al*. (2009) who also reported that *A. indica* contain alkaloids, flavonoids, cardiac glycosides, steroids, phenols, tannins, and saponins in significant concentration in the leaf extract. The methanolic extract of the leaves contain saponins, flavonoids, steroids and phenols while alkaloids, tannins, volatile oils, glycosides and terpenoids are absent. This corresponds with the report of Nataranjan *et al*., (2003) who reported that aqueous extract of *A. indica* contains only tannins, saponins, volatile oil, terpenoids and phenols.

The phytochemical analysis conducted on *D. viscosa* extracts showed that tannins, saponins, flavonoids, volatile oils, glycosides, phenols and terpenoids were detected in the methanolic extract. The aqueous extract contains only alkaloids, tannins, saponins, volatile oil, steroids, terpenoids and phenols. This result corroborates with that of Prakash *et al*. (2012) who reported tannins, saponins, flavonoids and terpenoids in the aqueous extract of the leaf. The petroleum ether extract contain alkaloids, tannins, saponins, flavonoids, volatile oil, steroids and phenols while glycosides and terpenoids are absent.

The methanolic extract showed highest degree of antibacterial activity against *Staphylococcus aureus* at its highest concentration of 50mg/ml. The least activity was against *Klebsiella pneumoniae*. However, *Escherichia coli* and *Pseudomonas aeruginosa* resisted the extract at all the concentration used*.* This result correspond with the work of Reddy *et al*. (2013); Mishra *et al*. (2013); Prashar *et al*. (2012) where *A. indica* leaf extract at different concentration inhibited the growth of *Staphylococcus aureus*.

Aqueous leaves extract of *A. indica* showed low to moderate activity where it produces its highest inhibition zone against *Klebsiella pneumoniae* while the least activity was against *Staphylococcus aureus*. *Escherichia coli* and *Pseudomonas aeruginosa* all resisted the extract*.* In an earlier study, De and Ifeoma (2002) also revealed that at a concentration of 10mg/ml, the crude extract of *Azadirachta indica* were unable to inhibit the growth of *Peudomonas aeruginosa* and *Escherichia coli*. The petroleum ether extract of *Azadirachta indica* inhibited the growth of all the organisms at all the concentrations used with the highest zone of inhibition against *Staphylococcus aureus* at concentration of 50mg/ml. The least activity was against *klebsiella pneumoniae*.

The methanolic leaves extract of *D.viscosa* inhibited the growth of the entire tested organism. It produced a higher zone of inhibition against *Salmonella typhi*. The least activity was against *Escherichia coli*. The aqueous extract of *Dodonea viscosa* was effective against all the test isolates though to a varying degree with the exception of *E.coli* and *P.aeruginosa*. The zone of inhibition was highest against *Klebsiella pneumoniae* while the least activity was on both *Staphylococcus aureus* and *Salmonella typhi* respectively. This result corresponds with that of Thring *et al.* (2007) where aqueous leaf extract of *D. viscosa* showed activity against *K. pneumoniae* and *P. aeruginosa*. The petroleum ether extract of *D. viscosa* showed the lowest activity on the test isolates with activity only against *Salmonella typhi*. The remaining test bacteria resisted the extract at various concentrations used. This was supported by the previous study performed by Lin *et al*. (1999) that had found that plant extracts inhibited the Gram-positive microorganisms more than the Gram-negative ones. The less sensitivity of *Escherichia coli* and *Pseudomonas aeruginosa* may be due to the presence of plasmid conferring resistance which corresponds with the work of (Lawal *et al*., 2012).

Synergistically, the antibacterial activity of the combined plant extract was less compared to the activity of the individual plant extracts as evidenced by the lower zones of inhibitions produced. The finding of the result therefore clearly indicates that the plants extracts combined had an antagonistic effect or activity against the tested pathogens. One possible reason could be that the phytochemicals of the two plants may have not interacted at all. This observation is in line with the work of Raj and Yogini (2015), who worked on comparative antimicrobial activities of neem (*Azadirachta indica)* and curry leaves (*Murraya koenigii*) extract and their synergistic effect against selected pathogenic bacteria and fungi and found out that no synergism was observed when the two plants were combined against the test bacteria and fungi Furthermore, it could be that the mixing of extracts meant a smaller quantity of extract dissolved in a larger volume of solvents and thus due to decreased concentration, the individual activities of the phytochemicals may have reduced.

## CONCLUSION

The study shows that the *Azadirachta indica* and *Dodonea viscosa* leaves extract contain chemical constituents of pharmacological significance. The plants had an antibacterial activity on the test isolates with the methanolic leaves extract of the two plants exhibiting the highest activity on the test isolates when compared to the aqueous and petroleum ether extract. However, no synergistic antibacterial activity was observed when the two plants extract were used together.

## RECOMMENDATIONS

The isolation and purification of bioactive compounds in this two plants extracts should be carried out to determine the specific active compound responsible for the antibacterial activity of the plant extracts against different organisms. More also, More research should be conducted to identify factors that are responsible for the lack of synergistic activity of the two plants.

**Conflict of interest**

The authors declared no conflict of interest.

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