



## ANTIBACTERIAL ACTIVITY OF *Dodoneae viscosa* LEAF EXTRACT AGAINST SELECTED ENTERIC BACTERIA

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### ABSTRACT

*Dodoneae viscosa* is a species of flowering and ornamental plant in *Sapindaceae* family, Australian in origin and known to be distributed in the tropical, subtropical and temperate regions of the world. The plant is used as a traditional medicine in different countries and its various infusions are used to treat many illnesses including sore throats, ulcers, diarrhea and malaria. This study determined the phytochemical components and also the antimicrobial activities of *Dodoneae viscosa* leaf extract against *Salmonella* spp and *E. coli* obtained from previous isolations in Microbiology laboratory of Federal University Dutse Jigawa state. The leaves were extracted using Soxhlet apparatus for ethanol extract and maceration method for aqueous extract after which the phytochemical components were determined using standard methods while agar well diffusion method was used to determine the antibacterial activity. Results of phytochemical screening showed that bioactive compounds including flavonoid, tannins, phenols and saponins were present in both extract. Growth of the organisms: *Salmonella* spp and *E. coli* were inhibited with Minimum Inhibitory Concentration of 50mg/ml and 25mg/ml respectively. The results of ethanolic extract had higher percentage yield of 23.6% compared to the aqueous extract with 16.46%. It further showed that the extract contain some potent phytochemical constituents which are known to be of therapeutic values and its antibacterial activity against the two selected enteric bacteria at 50mg/l and 25mg/l indicated its potency as antimicrobial agent hence, could be useful in the treatment of gastroenteritis in future and exploited in pharmaceutical industries for the production of antimicrobial agent.

**Keywords:** Antibacterial, Activity, *Dodoneae viscosa*, Pathogens, Leaf, Extracts, *Salmonella*

### INTRODUCTION

*Escherichia coli* and *Salmonella* spp are members of enteric bacteria which are known to be gram negative bacilli, facultative anaerobes, ferment glucose, catalase positive, oxidase negative, causing gastrointestinal diseases or present as flora (McMahon, 2014). *E. coli* is a normal flora of lower intestine of warm blood organisms but some strains could be pathogenic causing gastroenteritis, urinary tract infection and neonatal meningitis (Thompson 2007; Yu *et al.*, 2014). These infections were previously treated by the use of medicinal plants due to antimicrobial properties of the bioactive compounds present (Mohammad *et al.*, 2012). Teffo *et al.* (2010) and that of Khuram (2010) has shown that *D. viscosa* leaf extract has an immense effect on both *Salmonella* and *E. coli*.

Medicinal plants may be defined as those plants that are commonly used in treating and preventing specific ailments and diseases. They contribute significantly to livelihoods including the use as herbal medicine, and economically (Sospeter *et al.*, 2013). The use of herbs and medicinal plants in Africa for

therapeutic purpose has been a common practice but most of these plants and herbs are used without proper knowledge of their chemical constituents, spectrum of activity, inhibitory or bacteriocidal concentration (Saunders *et al.*, 1993; Evans, 1999). The investigation of efficacy of plant-based drugs has gained greater attention due to its little or no side effects, cost effective and availability (Chatterjee, *et al.*, 2011). In fact, the problem of emergence of microbial resistance to most synthetic antibiotics due to indiscriminate use has prompted the discovery of new antimicrobial agents in which plants have been highly instrumental (Gustavo *et al.*, 2010; Tarun *et al.*, 2012). Previous researchers have provided scientific basis for the use of medicinal plants as against infectious diseases (Kitula, 2007). In addition to these problems, antibiotics are sometimes associated with adverse effects on host, which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immune suppression and allergic reactions. Therefore, the search for new drugs from other sources, such as plants is increasingly becoming necessary.

According to Bhattacharjee, *et al.* (2010), the World Health Organization advocated traditional medicine as safe remedies for ailments of microbial and non- microbial origins. Thus there have been revivals of interest in herbal medicines. This is partly due to increase in awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular compounds from plant Kingdom. Plants have been identified as the basic source of modern medicine (Nair *et al.*, 2005).

*Dodonaea viscosa* is commonly used as traditional medicine in different countries with the stem or leaf infusion used to treat sore throats, root infusion to treat colds and the seeds in combination with other plants to treat malaria. The leaves are used to treat itching, digestive system disorders, including indigestion, ulcers and diarrhea and the powdered leaves to expel round worms (Rojas *et al.*, 1996; Ivan, 2001). The plant is also used as antibacterial and insecticidal agent ((Rojas *et al.* 1992; Malarvannan, *et al.*, 2008).

The plant is commonly known as Pribet in Hausa language, and Hop bush in English. It could be administered orally or as poultice to treat various ailments (Aliyu, 2006, Sandhya, 2009). The active principal constituents of *D. viscosa* is acid resin. The leaves contain two acid resins, gum, albumen, tannin, and ash. Studies conducted by Shaikh *et al.* (2010) have shown that the leaves contain carbohydrates, flavonoids, fixed oil, proteins and amino acids, saponins, steroids and sterols, tannins, and triterpenoids and also antimicrobial activity against both Gram-positive and Gram-negative organisms. This research study is aimed at determining the phytochemical and antibacterial activity of leaf extract from *D. viscosa* against *E. coli* and *Salmonella* spp.

## MATERIALS AND METHODS

### Study Area

The study area was in the old site of the Federal University Dutse, Jigawa state where this kind of plant are mostly found. The picture of the plant is shown in Fig 1 below.



Fig 1: Picture of *Dodonaea viscosa* from Federal University Dutse Jigawa state

### Collection of Samples

Fresh leaves of *D. viscosa* were collected from healthy and fully grown plants from the old site of the Federal University Dutse, Jigawa state. The leaves were detached from the plant during early morning hours and were washed with water and taken to Botany unit of the department of Biological sciences to confirm the identification after which they were taking to Microbiology

Laboratory in the Department of Microbiology and Biotechnology for analyses.

### Laboratory procedures

The leaves were air-dried by spreading them on a table for a period of two (2) weeks after which they were grinded to powder using pestle and mortar (Mukhtar and Tukur, 1999) and were sieved using 35mm sized mesh. Fifty gram (50g) of the powdered material was extracted with ethanol (95%) by using

soxhlet apparatus in the main laboratory of the department of Chemistry, Federal University Dutse. Also, the freshly prepared powdered was extracted in n-hexane at room temperature for 72 h using cold maceration method to carry out aqueous extraction.

#### Phytochemical screening

Phytochemical screening of both extract were carried out including test for reducing sugars, tanins, alkaloids, saponin, flavonoid, phenols, and volatile oil (Ciulci, 1994).

#### Identification of test organisms.

The selected test organisms *Salmonella* and *E. coli* were obtained from previous isolations in Microbiology laboratory, Federal University Dutse and were both confirmed using selective media such as eosine methylene blue agar for *E. coli*, MacConkey agar and salmonella-shigella agar for *Salmonella* spp. Relevant biochemical tests including Indole, motility, methyl red, Voges-proskers and citrate were carried out for further identification of the organisms (Cheesebrough, 2002).

#### Preparation of stock solution.

The stock solution was prepared using two-fold dilution method after which the various concentrations (50mg/ml, 25mg/ml, 12.50mg/ml and 6.25mg/ml) were prepared.

#### Antibacterial assay

A loop-full of overnight culture of each of the test organisms was inoculated into 10ml of prepared Mueller-Hinton broth and incubated at 37°C for 24 h. A 10-fold dilution was made as bacterial suspension and was standardized using 0.5 McFarland turbidity standard. This was used in agar well diffusion method previously described by (Nester *et al.*, 2004) for the determination of antibacterial activity of the plant extracts. A loop-full of the standardized bacterial suspension was evenly spread on the surface of a prepared dried nutrient agar plate. Thereafter, the wells were made by using sterilized cork borer. The diluted extracts (2ml) were then introduced into the wells and also another agar plate was prepared and ethanol was used instead as control. A disc (5µg) of ciprofloxacin was used on each plate as control for bacterial sensitivity.

The plates were incubated for 24 hours at 37°C after which the zone inhibition around each well were measured in millimeter (CLSI, 2015).

The Minimum Inhibitory Concentration (MIC) was determined using broth dilution method. This was done by adding 1ml of the extracts into the same amount of nutrient broth in a test tube and 1ml of the inoculum was added. The tubes were incubated at room temperature for 24 h after which the MIC was determined by visualizing the turbidity. This was taken as lowest concentration of the extracts in which there was no growth (Abalaka *et al.*, 2013)

The Minimum Bactericidal Concentration (MBC) of the extracts were determined by collecting 0.1ml of broth dilution from MIC without any turbidity and inoculated on freshly prepared nutrient agar plates. These were incubated at 37°C for 48 h taken as the concentration of the extract with the highest dilution that gave no growth on the nutrient agar plate (Cheesebrough, 2010).

## RESULTS

Table 1 shows the physical properties of the leaf extract obtained from *Dodoneae viscosa*. The colour was dark- green and turbid for ethanol extract and dark brown and oily for aqueous extract. Ethanol extracts of *D. viscosa* yielded more extract with a percentage yield of 23.60% compared to 16.46% aqueous extract.

In Table 2, the phytochemical screening carried out indicated that some bioactive compounds including flavonoids, alkaloids, tannins, phenols, saponins, volatile oils and reducing sugars, saponins, flavonoid, phenol, tannins were present in both extracts and volatile oil, alkaloid are present only in the ethanol extract. Furthermore reducing sugar was found in only aqueous extract.

The antibacterial properties of the extracts are shown in Table 3. The antimicrobial assay showed some degrees of activities against the isolates tested, with the highest activity shown against *Salmonella* at 50mg/ml and a zone diameter of 15mm.

**Table 1: Physical properties of *Dodoneae viscosa* leaf extract obtained.**

PROPERTIES	AQUEOUS	ETHANOLIC
Colour	Dark brown	Dark green
Texture	Oily	Turbid
Weight of plant leaf used (g)	50	50
Weight of extract recovered (g)	8.23	11.80
Percentage yield (%)	16.46	23.60

**Table 2: Phytochemical characteristic of the *Dodoneae viscosa* leaf extract**

TEST	AQUEOUS	ETHANOL
Alkaloid	-	+
Flavonoid	+	+
Saponins	+	+
Volatile oils	-	+
Tannins	+	+
Reducing sugar	+	-
Phenols	+	+

It was observed that antibacterial effect of both extracts against the test organisms were limited to the first two higher concentrations: 50mg/ml and 25mg/ml, below which no antibacterial effect of any of the extracts was observed. However, zone diameters of *Salmonella* spp were greater, showing that the antibacterial effect of the extracts was higher compared to that of *E. coli*

There was no any zone of inhibition observed in *E. coli* culture plate indicating none antibacterial action of the aqueous extract against the bacterium. In the case of ethanolic extract, zone of inhibition (13mm and 11mm) was only observed at 50 and 25mg/l respectively.

**Table 3: Antibacterial activities of the leaf extract of *Dodoneae viscosa* on the selected bacteria**

BACTERIUM	AQUEOUS EXTRACT				ETHANOLIC EXTRACT			
	Z(C)				Z(C)			
<i>Salmonella</i> spp	10 (50)	7 (25)	0 (12.5)	0 (6.25)	15 (50)	12 (25)	0 (12.5)	0 (6.25)
<i>E. coli</i>	0 (50)	0 (25)	0 (12.5)	0 (6.25)	13 (50)	11 (25)	0 (12.5)	0 (6.25)

Key: Z= Zone of inhibition (mm), C = Concentration of leaf extract (mg/ml).

Tables 4-7 show the Minimum Inhibitory Concentration (MIC) and consequently the Minimum Bactericidal Concentration (MBC) of each of the extracts.

**Table 4: Minimum Inhibitory Concentration of the Aqueous Extract**

TEST ORGANISM	Concentration of aqueous extract (mg/ml)			
	50.00	25.00	12.50	6.25
<i>E.coli</i>	+	+	+	+
<i>Salmonella</i> spp	MIC	-	+	+

MIC = Minimum inhibitory concentration, - = No turbidity, + = Turbid

**Table 5: Minimum Bactericidal Concentration of Aqueous Extract**

TEST ORGANISM	Concentration of aqueous extract (mg/ml)			
	50.00	25.00	12.50	6.25
<i>E.coli</i>	+	+	+	+
<i>Salmonella</i> spp.	MBC	-	+	+

MBC = Minimum Bactericidal concentration, - = No turbidity, + = Turbid

**Table 6: Minimum Inhibitory Concentration of Ethanolic Extract**

TEST ORGANISM	Concentration of ethanolic extract (mg/ml)			
	50	25	12.50	6.25
<i>Salmonella</i> spp.	-	MIC	++	+
<i>E.coli</i>	-	MIC	++	+

MIC = Minimum Inhibitory Concentration, - = No turbidity, + = slightly turbid, ++ = Highly turbid.

**Table 7: Minimum Bactericidal Concentration of Ethanolic Extract**

TEST ORGANISM	Concentration of ethanolic extract (mg/ml)			
	50	25	12.50	6.25
<i>Salmonella</i> spp.	MBC	-	++	+
<i>E.coli</i>	MBC	-	++	+

MBC = Minimum Bactericidal Concentration, - = Not detected, + = Slightly turbid, ++ = Highly turbid

## DISCUSSION

The findings showed that the leaf extract of *D. viscosa* had various compounds in the extract including alkaloids, flavonoids, saponins, volatile oils, tannins and phenols most of which have therapeutic values. Most of these were obtained in previous studies on medicinal plants including Mann *et al.* (2008), Bulus *et al.* (2008), Azeez *et al.* (2015), and Chiroma *et al.* (2018). The presence of these metabolites might be responsible for antibacterial activities exhibited by the extract. This is in line with the findings of Sofowora (1986), Araona and Ike (2017) and Chiroma *et al.* (2018) who explained that the presence of secondary metabolites in plant produce some biological activities in man and animal and this might be responsible for use of such plants as herb.

The inability of aqueous extracts to show significant antibiotic activity suggested high resistance rate of the bacteria against the extract or might be tied to incomplete extraction of the active component due to the use of water as solvent, insufficient active components to exhibit any antimicrobial activity (Elmahood *et al.*, 2005; Mbata *et al.*, 2008).

The findings in this study also showed higher degree of inhibition of the tested organisms with increased concentrations of the extracts, indicated the importance of the extract as an antimicrobial agent, containing more phytochemical constituents, bioactive ingredients and therefore potent antibacterial effect (Ahmed *et al.*, 2012; Jayashree, 2013). These results are in agreement with the findings of Rojas *et al.* (1992), Mothana *et al.* (2008) and Nasrullah *et al.* (2012). The antibacterial effect of the leaf extract against *E. coli* was in agreement with the result of Teffo (2006), Teffo *et al.* (2010) and that of Khuram (2010) which showed that *D. viscosa* leaf extract has an immense effect on both *Salmonella* and *E. coli*.

In this research, the MIC of 6.25mg/ml and 12.5mg/l is similar to that obtained by Aboaba *et al.* (2006) and Araona and Ike (2017). It was observed that the test organism *Escherichia coli* was less susceptible to ethanolic leaf extract, showed inhibition zone of 13.00mm and 11.00mm at the concentration of 50.00mg/ml and 25.00mg/ml respectively. This might be due to the presence of plasmids conferring resistance (Ivan, 2001; Neoji *et al.*, 2008).

## CONCLUSION

The leaf extract of *D. viscosa* has been confirmed in this study to contain some potent phytochemical constituents known to be of therapeutic values and could be useful in the treatment of gastroenteritis in future. The antibacterial effect against *Salmonella* spp was higher compare to *E. coli* which showed that extracts from *D. viscosa* could be more effective in the treatment of diseases initiated by them. The leaves of *D. viscosa* could be exploited in the production of antimicrobial agents in pharmaceutical industries.

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