UJPICR Volume 2 Number 1 : June, 2022
UMYU Journal of Pure and Industrial Chemical Research
Volume 2 No. 2 December, 2022.
ISSN: **2814 – 1628/2814 - 1636**
DOI: https://doi.org/10.55688/ujpicr22.009

Screening of Dodonaea Viscosa Linn (Sapindaceae) for Antibacterial and Cytotoxic Activities

Abubakar Sani*[1] and Oumar Al-Moubrak Adoum[2]

[1]Department of Pure and industrial Chemistry, Faculty of Natural and Applied Sciences, Umaru Musa Yar'adua University, Dutsin-ma Road, P.M.B,2218, Katsina, Nigeria

[2]Department of Pure and industrial Chemistry, Bayero University, Kano P.M.B. 3011, Kano, Nigeria

*Corresponding Author: abubakar.sani@umyu.edu.ng

ABSTRACT

The crude ethanol extract of *Dodonaea viscosa* Linn (Sapindaceae) was partitioned into chloroform, aqueous, ethyl acetate, methanol and n-hexane soluble fractions. The extract and five fractions were tested against the larvae of *Artemia saline* [Brine Shrimp Lethality Test (BST)]. The aqueous and methanol extracts were found to exhibited remarkable toxicity on brine shrimp larva at LC_{50} value of 0.1326 and 62.5 µg/ml, while no activity recorded in the ethanol, chloroform, ethyl acetate, and n-hexane fractions LC_{50} value >1000 µg/ml. The crude ethanol extract and five solvent fractions were tested for antimicrobial activity using agar diffusion method and the results revealed the zone of inhibition 15.0 mm at 10,000µg/ml was exhibited by the aqueous soluble fraction of the leaves of *Dodonaea viscosa* against *S. typhi*. Meanwhile, methanol and ethanol fractions of *Dodonaea viscosa* exhibited zone of growth inhibition 15mm and 30mm against *S. aureus*, at 10,000µg/ml.

Keywords: *Antibacterial activity*; *Cytotoxicity*; *Dodonaea viscosa* Linn

INTRODUCTION

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. Additionally, the disease is underestimated because there are no bacteriology laboratories in most areas of developing countries. These factors are believed to result in many cases going undiagnosed. From literature [3] and the incidence of typhoid fever recorded in control groups in large vaccine fields trials

with good laboratory support, it has been estimated that approximately 17million cases of typhoid fever and 600,000 associated deaths occur annually [7]. However, the prevalence of bacteraemia in febrile children is quite high [14]. It has been suggested that exposure to the bacteria is higher than indicated by the figures that are based solely on the clinical syndrome of typhoid fever in endemic areas [21]. Typhoid fever has been associated with man since close to the first appearance of mankind and it may have first infected human ancestors anywhere from 200,000 to 2million years, ago. The bacterium can survive in contaminated water, but does not have any host other than man [24].

There are over 1,000 different strains of the bacterium of which only a few cause typhoid. A closely related bacterium *Salmonella typhimurium* causes a similar disease in the mouse, but does not affect man and the infestation of the pathogen in the mouse is used as a model to study the disease. *S. typhi* attacks the tissues on the inner surface of the intestine which constitute the first line of defense against food and water- borne infections. It does not have the cell surface features that normally trigger a defensive response but it can fool the cells of the patches to take it in without attacking it. The infected tissue becomes inflamed resulting in diarrhea or constipation. There may be bleeding of the intestine leading to bloody stools. In severe cases, the disease may punch holes in the intestine leading to peritonitis infection of the abdominal cavity and death [6].

Medicinal plants played an important role in curing various kind of ailments particularly in African sub region, where nature provides tremendous reservoir of plants with secondary metabolites. If these plants are properly harness, this could probably help immensely in health care delivery systems in

Africa as an alternative source of medication [11].

Dodonaea viscosa Linn (Sapindaceae) commonly called ‘‘Pribet or Fulawa’’, in Hausa, is an evergreen dioecious or monoecious multi-stemmed shrub or single stemmed small tree up to 7-9 m tall. Most part of young branches are greenish and prominently angled [13]. It is widely spread in tropical and sub-tropical regions [8]. The leaves of *D. viscosa* are used to relieve itching. However, digestive system disorders, such as indigestion, ulcers, diarrhea and constipation are commonly treated with decoction of either the leaves or roots of the plant. The stems and leaves are also used to treat fever while the seeds of *D. viscosa* are reported to treat malaria [2]. The leaf juices are used for the treatment of Trachoma and powdered leaves are given to expel round worms. The stem or leaf infusions are used to treat sore throats; root infections to treat colds, and also stimulate milk production after giving birth and to treat dysmenorrhoea and irregular menstruation [2].

Bioactivities on extracts of the plant have been investigated and reported in literature. Aqueous and ethanol extracts were found to exhibit cardiac depressant and coronary-constricting properties. Extracts from the seeds was shown to have phagocytosis enhancing, analgesic and molluscicidal activity [2]. Chemical compounds such as quercetin, kaempferol and rutin have been identified in *D. viscosa*. All were reported present in the seed, bark, inflorescence and leaves of *D. viscosa*. Other compounds like aliarin, dodonic acid, viscosol [20] stigmasterol, isorhamnetin [17], [18], penduletin, quercetin, doviscogenin [9] dodonosides A and B have been isolated from *D. viscosa* [25].

The leaves were reported to possess local anaesthetic, smooth muscle relaxant [19], antibacterial [6], antifungal [13], anti-

inflammatory [5], and anti-ulcergenic activity [23]. It had been reported that 95% ethanol extract of *D. viscosa* leaves showed anti-ascariasis, anthelmintic, cardiac depressant, hypotensive, uterine relaxation and vasoconstrictor activities in different experimental models. The reported efficacy of this plant in the cure of such infectious diseases has attracted our attention to further establish more scientific basis for their application [22].

This study screened the extracts obtained from the leaves of *Dodonaea viscosa Linn* against some selected microbes and *Artemmia salina* for their respective antimicrobial and cytotoxicity potentials.

Materials and Methods

Collection of Plant Materials

The plant materials used in this study were collected in June, 2007, from Yako village in Kiru Local Government Area of Kano State, Nigeria. The plants were identified by Malam Baba Ali Garko (Staff of Bayero University, Kano), and authenticated by Mr. Mohammad Musa of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria. A voucher specimen (No 900241) has been deposited at Herbarium.

Extraction of Plant material

The air-dried and grounded plant sample (200g) were extracted with absolute ethanol (700ml) at room temperature for two weeks. The percolates were evaporated to dryness *in vacuo* to afford a residue coded (F001) [1].

Fractionation of crude extract

The crude extract (F001) was solvent partitioned to give chloroform (F002), water (F003) and ethyl acetate (F004) soluble fractions. The chloroform soluble fraction (F002) was further partitioned between n-hexane and methanol to give n-hexane (F005) and methanol (F006) soluble

fractions (Table1). All the fractions were concentrated *in vacuo*, weight of the fractions was recorded and stored in a freezer [4].

BRINE SHRIMP LETHALITY BIOASSAY

A brine shrimp lethality (BST) bioassay is capable of detecting a broad spectrum of bioactivity present in crude extracts. The technique is easily mastered, costs little and utilizes small amount of test materials. The bioassay provides a front-line screening that can be backed up by more specific and more expensive bioassays once the activity has been detected [15].

The plant extract was screened against brine shrimp larvae of Artemia saline according to the method described [4]. In this test, sea water obtained from Lagos Beach, Nigeria was used to culture the Artemia larvae. To enhance the solubility of test, dimethylsulphoxide was added to test materials and control vials. The results obtained are depicted in Table2. The extent of toxicity of a plant extract bioactivity is estimated using the Lethal concentration (LC_{50}) value of the mortality of the brine shrimp larvae using Finney program. The lower the LC_{50} values in µg/ml the more efficacious as drug it could be [11].

ANTIMICROBIAL BIOASSAY

Sources of Microorganisms

Pure cultures of *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* were obtained from the Microbiology Laboratory, Aminu Kano Teaching Hospital, Kano, Nigeria. The three bacterial cultures were maintained in nutrient agar slant at 4 °C before use.

Preparation of Inocula

The inoculum was prepared from the stock cultures which were maintained on nutrient agar slant at 37 °C overnight and sub-cultured in nutrient broth using a sterilized

wire loop and incubated at 37 °C for 24 hours. The density of suspension to be inoculated was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (1% v/v).

Preparation of Sensitivity Disc

A paper puncher was used to prepare discs of about 6mm diameter from whatman's No 1 filter paper. Batches of 100 discs were transferred into Bijou bottles and sterilized in the oven at 110 °C for 24 hours. The stock solution of 10mg/ml of the plant extract for the bioassay was prepared by dissolving 0.01g of each fraction of *D. viscosa* in 1ml Dimethyl sulfoxide (DMSO) (i.e 10,000µg/ml). Three concentrations of 5000, 2000, and 1000µg/ml were prepared by dissolving 0.5ml. 0.2ml and 0.1ml of the stock solution into 0.5ml, 0.8ml and 0.9ml of DMSO, respectively. One milliliter (1ml) of the extract from 10,000µg/ml, 5000µg/ml, 2000µg/ml and 1000µg/ml concentrations were each transferred into separate bottles containing 100 discs. Since each disc can absorb 0.01ml, the four bottles yielded discs of 100µg/disc, 50µg/disc, 20µg/disc, and 10µg/disc, respectively.

Antibacterial Susceptibility Test

Disc agar diffusion method was employed for antibacterial assay. Four concentrations 100µg/disc, 50µg/disc, 20µg/disc, and 10µg/disc for each fraction of *D. viscosa* extract were prepared. A sterile wire loop loaded with standard culture was used in streaking agar plates distributed evenly and aseptically in an inoculation chamber. A standard antibiotic disc Ciprofloxacin (30µg, control disc) were aseptically pressed firmly at the center using sterile forceps unto the inoculated plates. The zone of inhibition (in diameter, mm) was measured to the nearest whole number using a transparent meter ruler (Table 3).

Results and Discussions

Physical parameters (weight, colour and texture) of the crude extract and various factions from the leaves of *Dodonaea viscosa* Linn were presented in (Table 1). The results of the preliminary BST screening of solvent partitioned extracts of the leaves of *Dodonaea viscosa* Linn

showed that about 33% of the plant extracts were active in BST (Table 2). The bioassay showed that aqueous soluble fraction (F003) of *Dodonaea viscosa* LC_{50} = 0.1326 (Table 2) exerted highest lethal activity. The lowest activity was found in the methanol soluble fraction (F006) LC_{50} =62.5µg/ml. Meanwhile, the ethanol (F001), chloroform (F002), ethyl acetate (F004), and n-hexane (F005) soluble extracts have BST at LC_{50} values greater than 1000µg/ml and are therefore not active on brine shrimp. This may partly be the reason why some shrimps prefer to associate with the plants in their sea environment. These results suggest potency in the plants extracts under investigation.

Table 1: Physical parameters of the crude and various fractions of *Dodonaea viscosa* Linn leaves extracts

Fraction	Weight(g)	Colour	Texture
F001	34.00	Greenish	Oily
F002	5.90	Brownish	Gummy
F003	1.80	Brownish	Liquid
F004	1.30	Greenish	Solid
F005	1.60	Greenish	Solid
F006	0.90	Greenish	Gummy

Key: F001 = crude ethanol extract; F002 = chloroform soluble fraction; F003 = aqueous fraction; F004 = ethyl acetate soluble fraction; F005 = n-hexane soluble fraction; F006 = methanol soluble fraction.

Table 2: The BST activity of the crude and various fractions of *Dodonaea viscosa* Linn

Fractions	**LC_{50} µg/mL**	**Remarks**
F001	>1000	Inactive
F002	>1000	Inactive
F003	0.1326	Active
F004	>1000	Inactive
F005	62.50	Active
F006	>1000	Inactive

LC_{50} is determined at 95% confidence interval.

Table 3: Antimicrobial activity of the ethanol extract of *Dodonaea viscosa* Linn leaves

Isolates	**Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)**			
1000	**2000**	**5000**	**10000**	**control**
Staphylococcus aureus	07	09	13	30
30				
Salmonella typhi	00	07	10	16
30				
Escherichia coli	08	10	12	13
34				

Table 4: Antimicrobial activity of the chloroform fraction of *Dodonaea viscosa* Linn leaves

Isolates	Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)				
	1000	**2000**	**5000**	**10000**	**control**
Staphylococcus aureus	00	00	10	13	20
Salmonella typhi	00	00	07	14	32
Escherichia coli	00	07	08	13	30

Table 5: Antimicrobial activity of the aqueous fraction of *Dodonaea viscosa* Linn leaves

Isolates	Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)				
	1000	**2000**	**5000**	**10000**	**control**
Staphylococcus aureus	07	09	11	15	26
Salmonella typhi	08	11	12	15	40
Escherichia coli	00	07	10	13	35

Table 6: Antimicrobial activity of the ethyl acetate fraction of *Dodonaea viscosa* Linn leaves

Isolates	Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)				
	1000	**2000**	**5000**	**10000**	**control**
Staphylococcus aureus	00	09	10	12	30
Salmonella typhi	00	08	10	12	25

Escherichia coli 32	00	08	10	13

Table 7: Antimicrobial activity of the methanol fraction of *Dodonaea viscosa* Linn leaves

Isolates	Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)			
	1000	2000	5000	10000	control
Staphylococcus aureus 30	00	09	10	12
Salmonella typhi 25	00	08	10	12
Escherichia coli 32	08	08	10	13

Table 8: Antimicrobial activity of the n-hexane fraction of *Dodonaea viscosa* Linn leaves

Isolates	Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)			
	1000	2000	5000	10000	control
Staphylococcus aureus 28	00	10	13	15
Salmonella typhi 32	00	10	11	14
Escherichia coli 30	07	09	11	13

Key: Zone of inhibition for disc = 6mm

The antibacterial activities were carried out on all the fractions obtained as shown in the tables 3 to 8. It was reported that susceptibility of bacterial culture to extract was determined by measurement in the following ranges; 0-7 mm indicates inactive; 8-11 mm indicates weak activity while 12

mm and above indicates strong activity. However, the zone of inhibition 15.0 mm at 10,000µg/ml was exhibited by the aqueous soluble fraction of the leaves of *Dodonaea viscosa* against *S. typhi*. The potency of the aqueous fraction was comparable to the standard antibiotics (i.e ciprofloxin) used. The ciprofloxin have a universal activity against the three test organisms *S. aureus, S. typhi* and *E.coli* with zone of inhibition ranging from 12.0 to 40.0 mm respectively. Methanol and ethanol fractions of *Dodonaea viscosa* exhibited zone of growth inhibition 15mm and 30mm against *S. aureus,* at 10,000µg/ml.

CONCLUSION

Based on this work, the leaves of *Dodonaea viscosa* Linn, provides a scientific basis for the ethnomedicinal uses of the plant in the Northern region of Nigeria to cure typhoid fever. The zones of inhibition exhibited by the methanol and chloroform fractions of *Dodonaea viscosa* on *staphylococcus aureus* justified their uses by traditional medicinal practitioners in the treatment of sores, bores, and open wounds. The cytotoxic activity observed on the aqueous, and methanol extracts of *Dodonaea viscosa* may lead to the discovery of new cytotoxic compounds. The extracts should also be evaluated for the pesticide activity. Further research to detect and characterize bioactive compounds of the leaves of *Dodonaea viscosa* needs to be carried out.

Acknowledgement

Our gratitude to Prof. M. D. Mukhtar of Biological Science Department, Bayero University Kano.

References

1. Adoum, O.A., Dabo, N.T. and Fatope, M.O. Bioactivies of some Savanna Plants in the Brine Shrimp Lethality Test and *In-vitro* Anti-microbial assay. *Inter. J. of Pharmacognosy*, 1997, 35 (5): 334 –337.
2. 2. Aliyu, B. S. Some enthnomedicinal plants of the Savanna regions of Africa. Description and Phytochemical, 2006, Vol. II Pp4.
3. Edelma, R., Levine Myron M. Summary of an International Workshop on typhoid fever. Reviews of infectious diseases, 1986, 8 (3) 329-349.
4. Fatope, M.O., Ibrahim, H. and Takeda, Y. Screening of higher plants reputed as pesticides using the brine shrimp Lethality assay: Inter. J of pharmacognosy, 1993, 31 (4): 250-256.
5. Getie M., Gebre-Marian T., Reitz R., Hohne C., Huschka C., Schimdtke M., Abate A., Neubert-R.H.H. Evaluation of the anti-microbial and anti-inflammatory activities of the medicinal plants Dodonaea viscosa, Rumex nervosus and Rumex abyssinicius J. of Fitoterapia, 2003, 74: 139-143.
6. Hernandez L., Rojas A. Pereda M.R. Mata R. Screening for antimicrobial activity of crude drug extracts and pure

natural products from Mexican medicinal plants.J. Ethnopharmacol.,1992, 35: 275-283.
7. http://www.cbwinfo.com/ Biological & pathogens/ ST. html08/08/2007, 5:25pm.
8. Ivanoff, B. N., Levine, M. M. and Lambert, P.H. Vaccination against typhoid fever; present status. Bulletin of the World Health Organization, 1994, 76 (6): 957-971.
9. Keay. Nigerian Trees. Vol II, Oxford: Clarendon Press,1964, pp 69.
10. Khan M.S.Y., Shamshad A.,and Jain P.C. Chemical Investigation of root bark of Dodonaea viscosa Linn. J. Nat. Prod. 1988, 4:12-13.
11. Mudi, S.Y., and Ibrahim, H. Brine Shrimp Lethality and Antimicrobial Susceptibility tests from the leaf extracts of Bryophyllum pinnatum and Cassia occidentalis on respiratory tract infections causing bacteria. Being a paper presented at the 31st Annual international conference and exhibition of chemical society of Nigeria (Deltachem 2008), At petroleum training institute, Effurum-Warri. Under the theme: Strategic Re-positioning of Chemistry for National development. 22nd -26th September, 2008, 1-13.
12. Mudi, S. Y., Salisu, A., and Sabiu, A. Phytochemical Screening and antimalarial activity of extracts from the stem bark of Adansonia digitate, 2012, 8 (1): 49-53.
13. Naovi, S.A.H., Khan M.S.Y., Vohora S.B. Antibacterial, antifugal and anthelmintic investigations on Indian medicinal plants. Fitoterapia 1991, 62: 221-228.
14. Pearman, G. Plant Resources of Tropical Africa. PROTA Programme, Wageningen, the Netherlands. 2002 Pp72-75.
15. Pisutthanan, S., Plianbangchang, P., Pisutthanna, N., Ruanruay, S. and Muanrit, O. Brine Shrimp lethality activity of Thai Medicinal plants in the family Meliaceae. Naresuam Univ. J. 2004, 12 (2): 13-18.
16. Punjabi, N.H. Cost evaluation of typhoid fever in Indonesia. Med. J. of Indonesia, 1998, 7 (5): 90 –93.
17. Rao V. K. Chemical Examination of the leaves of Dodonaea viscosa. J. Indian Chem. Soc. 1962, 39: 561-562.
18. Ramachandran N., Subramanian A.G., Sankara S. Isorhamnetin and quercetin glycosides from Dodonaea viscosa and Sapindus emarginatus. Indian J. chem. 1975, 13:639-640.
19. Rojas A., Cruzs. Porice-Montr H. Mata R. Smooth muscle relaxing compounds from Dodonaea viscosa. J.Plant Medica, 1996, 62: 154- 159.
20. Sachdev K., Kulshretha D.K., Viscosal A. C-3' prenylated flavonoid from Dodonea viscosa. Phytochemistry. 1986, 25: 1967-1969.
21. Saha S., Baqai A. H Hanif M., Darmstadt G. L., Ruhulamin M., Nagatake T., Santosham M., Black R. Typhoid fever in Bangladesh; implication for vaccination policy. The pediatric infectious disease journal, 2001, 20:521-524.
22. Sukkawala V.M., Desai. B. Physiological activity of the leaves of Dodonea viscosa. J.Sci. Ind. Res. 1962, 21:349-351.
23. Wain, J. Hendriksen, R. S., Mikoleit, M. L. Typhoid fever, Lancet, 2015, 387 (9973): 1136-1145.
24. Wagner H. Ludwig C., Grotjahn L., Khan M.S.Y. Biologically active Saponins from Dodonaea viscosa. J. of Phytochemistry, 1987, 26:687-701.