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Phytochemical Profiling and Antibacterial Activity of Ethanolic Extract of *Dodonaea viscosa*

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ABSTRACT

The emergence of antibiotic-resistant bacterial infection necessitates the exploration of novel phytotherapeutic agents. This study profiled the ethanolic extract of Dodonaea viscosa leaves and evaluate its antibacterial activity against Salmonella Typhi using maceration method. Phytochemical screening revealed the presence of bioactive compounds, including alkaloids, flavonoids, glycosides, tannins, terpenoid and phenolic compounds. Several conducted analysis techniques are extraction, isolation and structural illucidation to identify several phytocompounds and bacterial using dilution based tests with potential antibacterial properties. The extract revealed the presence of 6 secondary metabolites while results from thin layer chromatography shows a trace of secondary metabolites separated based on their RF value. The antibacterial analysis shows high zone of inhibition at second medium with 13 mm at 100 mg/ml while minimum inhibitory concentration (MIC) at 50 mg/ml and a minimum bactericidal concentration (MBC) at 100 mg/ml. The UV-Vis spectrophotometer analysis of the crude extract of Dodonaea viscose leaves was performed over a wavelength range of 200-850 nm, and 327 different compounds were found. These findings suggest that the ethanolic extract of D. viscosa possesses potent antibacterial properties, warranting further investigation as a potential phytotherapeutic agent against Salmonella Typhi and other antibiotic-resistant bacterial infections.

Keywords:
Extract,
Infection,
Activity,
Inhibition

INTRODUCTION

The knowledge of individual chemical constituents of a medical plant is essential for optimizing extraction procedures, understanding pharmacological activity as well as potential toxicity. Finding healing powers in plants is an ancient idea. People in all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory. Treating skin disorders with traditional medicine remains vital even in industrialized countries (Getie, 2000). A number of plants are used as traditional medicines in Ethiopia for the treatment of several skin diseases among which eczema, psoriasis and fungal infections are included (Abebe & Ayehu, 1993; Alemayehu, 2001). However, neither the safety nor the effectiveness for their claimed use is proved, and often the technique of preparation employed by traditional healers is generally poor and in most cases does not comply with the requirements of modern pharmacy practice (Getie, 2000; Popata *et al*., 2001).

In the last few decades, there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and fewer side effects (Ahmed, 2017). 75% of the world's population used plants for therapy and prevention (WHO, 2019).

The leaves are used to relieve itching, fevers, swellings, aches and can be used as antispasmodic agent, leaves and roots as painkiller to soothe toothaches and headaches, and lotion made from unspecified plant parts to treat sprains, bruises, burns and wounds. Trachoma is treated with applications of leaf juice, and powdered leaves are given to expel roundworms. Pulverized roots are a component of antihelmintic preparations (Aliyu e*t al.*, 2006).

The plant is also used as an antibacterial (Rojas *et al.*, 2019) and also has an insecticidal activity (Malarvannan e*t al.*, 2008). The active principle constituents of *D. viscosa* is an acid resin, Leaves contain two acid resins, gum, albumen, tannin, and ash. Study of leaves yielded -carbohydrates, Flavonoids, fixed oil, proteins and amino acids, saponins, steroids and sterols, tannins, and triterpenoids.

Salmonella spp. is important zoonotic pathogens and is considered one of the most common causes of food-borne illness in humans (Amand *et al.*, 2013). *Salmonella* is a gram negative bacillus and divided over 2,500 different serotypes. Some *Salmonella*

serovars can affect multiple host species and it makes a serious problem according to the food chain (Jung *et al.*, 2011).

Dodonaea viscosa, a medicinal plant with documented traditional uses, has shown promising antimicrobial potential; however, its antibacterial properties remain insufficiently explored. Existing studies are limited by their focus on a narrow spectrum of bacterial strains, methodological inconsistencies in extraction and assay protocols, and a lack of clarity regarding the mechanisms underlying its bioactivity. Addressing these gaps, the present study aims to profile the ethanolic extract of *D. viscosa* with respect to its antibacterial activity, thereby providing critical insights for the development of novel phytotherapeutic agents against antibiotic-resistant infections. Specifically, the work involves ethanol-based extraction of phytocompounds from *D. viscosa* leaves, phytochemical screening coupled with thin-layer chromatography (TLC), evaluation of antibacterial efficacy through minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays, and characterization of bioactive constituents using Fourier-transform infrared spectroscopy (FTIR) and UV-Visible spectrophotometric analysis.

Taxonomic Classification.

Kingdom: Plantae, Subkingdom: *Viridiplantae, Infrakingdom:Streptophyta,* Superdivision: *Embryophyta*, Division: *Tracheophyta*, Subdivision: *Spermatophytina*, Class: *Magnoliopsida*, Superorder: *Rosanae*, Order: *Sapindales*, Family: *Sapindaceae*, Genus: *Dodonaea*, Specie: *Dodonaea viscosa* (ITIS).

Fig 1 *Dodonaea viscosa*'s Plant

MATERIALS AND MEODS

Materials: The laboratory materials including chemicals and reagents were of analytical grade, which including: Test Tubes, Beakers, Measuring Cylinder, Volumetric Flasks (50 ml, 100 ml, 250 ml and 500 ml), Reagent Bottles, Droppers, TLC Set-Up, Analytical Weigh Balance, 20 ml Pipettes.

Plant Collection: fresh leaves of *Dodonae Viscosa* were sample-collected at the frontage of Prof Abubakar Saulawa Auditorium (Old) by exactly 4:47pm in Federal University of Dutsinma, Katsina State. The plant material samples of *D. viscosa* were surface-rinsed with tap water then with distilled water to remove surface dust and other solid contaminants. They were then dried in the shade and milled to a fine powder using mortal and pestle.

Extraction Method: Merceration method was adopted in this reseach work using 950 ml absolute ethanol at room temperature in chemistry laboratory, Federal University Dutsin-Ma. Katsina State. The extracted mixture were filtered and desolvatized using evaporation method without any external means.

Thin Layer Chromatography

Thin layer chromatography involves three steps; spotting the plate, developing it in solvent and visualizing spots corresponding to constituents. Spotting involves the use of a micro pipette to transfer a minute amount of a dilute solution at one end of the TLC plate. Thin layer chromatography plates are usually composed of a thin layer of silica gel coated onto a metallic or glass sheet. In the next step of development, the end of the TLC that has been spotted is placed into a shallow pool of solvent, which moves up the plate.

Bacteria Culturing: The bacterial isolate were collected from Microbiology Laboratory in Federal University Dutsin-Ma.

Anti-Bacterial Analysis

Anti-bacterial analysis of the crude extract sample of *Dodonaea Viscoasa* against *Salmonella Typhi* bacteria

were conducted by serial dilution using DMSO as a polar solvent. 100 mg/ml, 50 mg/ml and 25 mg/ml W/V of the *D. viscosa* crude extract concentration were prepared and tasted against *Salmonella Typhi.* The analysis was simultaneously conducted using two same medium (petri dishes containing 25 ml W/V solidified solution of nutrient broth each).

FTIR: The crude sample was taken to Umaru Musa University,. Katsina State. Nigeria.

For the functional group elucidation.

Result of Antibacterial Analysis:

Table 1: Table Representation of Zone of Inhibition (mm) Analysis

Extract	Solvent	Bacterial Isolate	Conc. of The Extract(mg/ml)			Control
			100	50	25	(mm)
Dodonaea viscosa	Ethanol	Salmonella	12 mm	11mm	00 mm	35
Dodonaea viscosa	Ethanol	Salmonella	13 mm	12 mm	00 mm	32

In both testing media, the highest concentrations of the extract demonstrated appreciable inhibitory effects against Salmonella Typhi (*S. Typhi*), while the lowest concentration showed no activity. Specifically, in the first medium, a 100 mg/mL extract produced a zone of inhibition measuring 12 mm, and 50 mg/mL yielded an 11 mm zone, whereas the 25 mg/mL concentration was entirely ineffective. The second medium showed a slight enhancement: 100 mg/mL produced a 13 mm zone and 50 mg/mL a 12 mm zone, with 25 mg/mL again showing no observable inhibitory activity. Ciprofloxacin was included in both assays as a positive control to validate the methodology. These results indicate a clear dose-dependent antibacterial effect of the crude extract against S. Typhi. Notably, comparable studies have reported similar efficacy: for example, methanol and ethanol extracts of Azadirachta indica (Neem) exhibited zones of inhibition ranging from 20 to 25 mm against S. Typhi, surpassing those observed for erythromycin controls (13-14 mm) (Anibijuwon & Udeze, 2009).

Result of Minimum Inhibitory Concentration (MIC):

Table 2: Table Representation of MIC Analysis

Extract	Solvent	Bacterial Isolate	Mic (mg/ml)
Dodonaea viscosa	Ethanol	Salmonella	50

The MIC of this research work for *Dodonaea viscose* crude extract against *Salmonella Typhi* is 50 mg/ml. similar results were found by (Alviano *et al*, 2008) with four Brazilian medicinal plants, where lower MIC values were noted for the periodontal pathogens compared to the cariogenic bacteria. Rios (2018) demonstrated that natural crude extracts which have the ability to exhibit antimicrobial activity at concentrations lower than 100 µg/ml are most likely to possess great antimicrobial potential due to the fact that the active compounds can be isolated and used at lower concentrations.

Result of Minimum Bactericidal Concentration (MBC):

Table 3: Table Representation of MBC Analysis

Extract	Solvent	Bacterial Isolate	MBC (mg/ml)
Dodonaea viscosa	Ethanol	Salmonella	100

RESULTS AND DISCUSSION

Preliminary phytochemical screening analysis is considered highly significant because crude drugs are known to contain a wide aarray of secondary metabolites that contribute to their pharmacological properties. The initial screening provides valuable insights into the chemical nature of the substances, highlighting the presence of diverse bioactive compounds essential for therapeutic applications (Maria *et al.*, 2009).

The minimum inhibitory concentration (MIC) of *Dodonaea viscosa* crude extract against Salmonella Typhi was found to be 50 mg/mL, indicating a moderate level of antibacterial activity. This finding aligns with observations by Alviano et al. (2008), who reported variable MIC values for extracts of four Brazilian medicinal plants, noting that periodontal pathogens were generally more susceptible than cariogenic bacteria. Although the MIC recorded in the present study is higher compared to the threshold proposed by Rios (2018), who suggested that crude extracts exhibiting activity at concentrations below 100 µg/mL possess strong antimicrobial potential, the extract's ability to inhibit S. Typhi at 50 mg/mL still reflects notable bioactivity. Such results suggest that the active compounds present in *D. viscosa* may be effective once isolated and concentrated, as the observed antibacterial effect could be attributed to phytochemicals such as flavonoids, saponins, or tannins, which are commonly associated with antimicrobial properties.

The crude extract of *Dodonaea viscosa* showed concentration-dependent antibacterial activity against Salmonella Typhi. At 100 mg/mL, inhibition zones ranged from 12-13 mm, and at 50 mg/mL, from 11-12 mm, while 25 mg/mL showed no effect. Ciprofloxacin served as the positive control.

The MIC of the extract was 50 mg/mL, indicating moderate efficacy, while the MBC was 100 mg/mL, confirming its bactericidal action. These findings align with Alviano et al. (2008), who reported similar patterns in Brazilian medicinal plants, though the MIC observed here is higher than the threshold suggested by Rios (2018) for strong antimicrobial potential (<100 µg/mL). Despite this, the extract demonstrates promising activity, suggesting potential for enhanced potency after purification.

Phytochemical studies by Amabeoku et al. (2001) identified flavonoids, alkaloids, saponins, tannins, and reducing sugars in *D. viscosa*. Flavonoids likely contribute most to its antibacterial effect, as these phenolic compounds are well known for antimicrobial and antiviral properties (Dixon et al., 1983). The results support the traditional use of *D. viscosa* and emphasize the need for further studies on bioactive compound isolation and synergy with conventional antibiotics.

Result of UV-Vis Spectrophotometric Analysis:

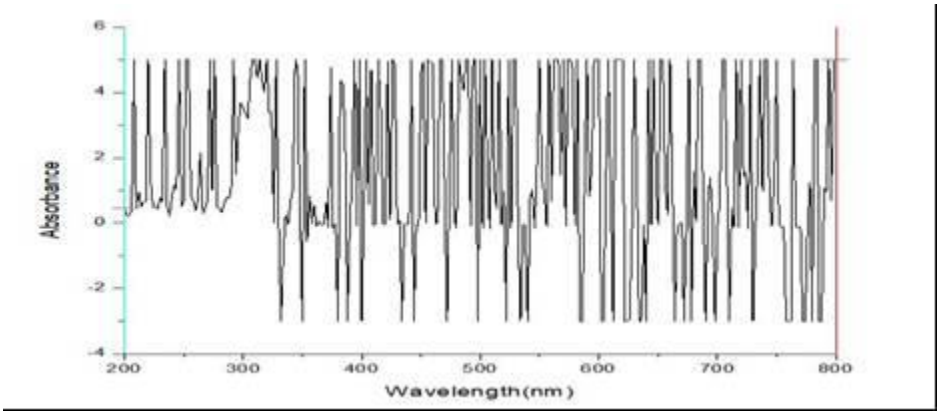

Fig 2: A Plot of UV-Vis Spectrophotometer Spectrum

The UV-Vis spectrophotometer analysis of the crude extract of *Dodonaea viscose* leaves was performed over a wavelength range of 200-850 nm, and 327 different compounds were found.

200–280 nm: Aromatic Compounds and Phenolic Acids.

The high absorbance in this region indicates the presence of aromatic compounds, such as phenolic acids and flavonoids. These compounds are known to exhibit strong absorption in the UV region due to their conjugated double bond systems.

280–320 nm: Terpenoids and Carotenoids

The presence of a shoulder or a small peak in this region may indicate the presence of terpenoids or carotenoids. These compounds absorb in the UV-Vis region owing to their chromophoric groups.

320–400 nm: Flavonoids

The absorbance in this region may be attributed to the presence of flavonoids, which are known to exhibit absorption in the visible region, particularly in the UVA band, due to their polyphenolic structures.

400–500 nm: Chlorophyll Derivatives and Other Pigments

The presence of a small peak or shoulder in this region may indicate the presence of chlorophyll derivatives or other pigments, such as anthocyanins, which absorb strongly in the visible region.

500–850 nm: Low Absorbance Region

The low absorbance in this region indicates the absence of significant amounts of pigments or compounds that absorb in the visible to near-infrared spectrum.
The UV-Vis spectrophotometer analysis of the crude extract of *Dodonaea viscose* leaves indicates the presence of a complex mixture of compounds, including phenolic acids, flavonoids, terpenoids, carotenoids glycosides, alkaloids, tannins etc and other primary metabolites such as amino acids, carbohydrates, organic acids and nucleotides.

Result of Phytochemical Screening:

Table 1: Phytochemical Content of *Dodonaea viscosa*

s/n	Test for	Reagent	Appearance	Inference
1	Alkaloids	Mayer's	Yellow	+
2	Glycosides	Borntrager's	Red	+
3	Phenolic compounds	Lead Acetate Solution	Yellow ppt	+
4	Flavonoids	Alkaline Solution	Orange	+
5	Saponins	Water	Persistent froth	-
6	Tannins	Ferric Chloride	Green	+
7	Terpenoids	Libermann-Burchard	Blue	+

The phytochemical screening reveaveled the presence of 6 secondary metaboilites including Alkaloids, Glycosides, Phenolic compounds, Flavonoids, Tannins and Terpenoids.

Result of Thin Layer Chromatography (TLC):

Table 2: Table Representation of Thin Layer Chromatography (TLC) Analysis

Spot	Distance Travelled By Component (Cm)	Distance Travelled Solvent (Cm)	By Retention Factor (R_f) 10% EA In n-hexane
E1	0.3	4.7	0.06
E2	0.5	4.7	0.11
E3	0.9	4.7	0.19
E4	1.2	4.7	0.26
E5	1.6	4.7	0.34
E6	2.0	4.7	0.43

Separation of the six compounds was achieved based on their interaction with the solvent system and the silica gel stationary phase. Since most compounds were colorless, the developed TLC plate was visualized under ultraviolet (UV) light at 366 nm. The retention factor (Rf) value, representing the ratio of the distance traveled by the compound to that of the solvent front, was calculated to characterize each component. These values were compared with those of reference standards for compound identification (Umass, 2016). A solvent mixture of 10% ethyl acetate in n-hexane proved optimal for separation, while silica gel-coated aluminum sheets served as the stationary phase. This system enabled the detection of six distinct spots under UV illumination, and the observed coloration indicated the presence of secondary metabolites.

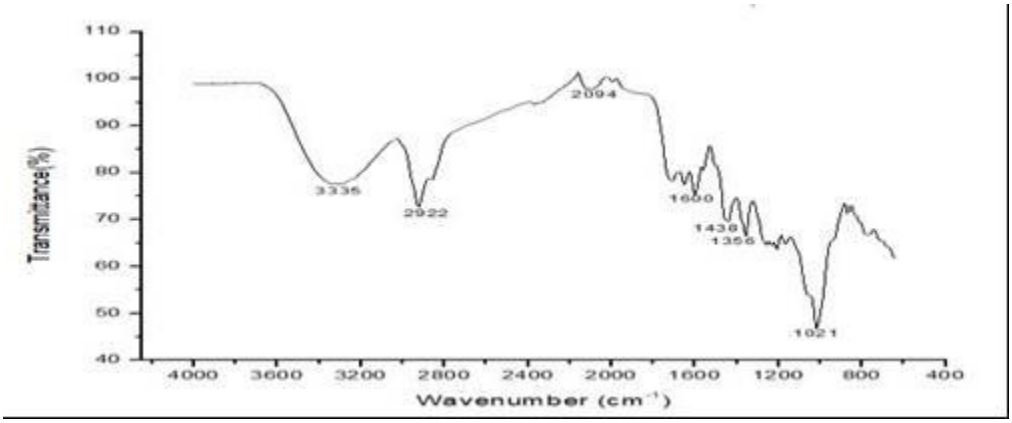

Fig 3: A plot of FTIR Spectrum

The FTIR spectrum revealed the presence of multiple functional groups indicative of an organic matrix with polar characteristics. A broad band near 3335 cm^{-1} corresponded to hydrogen-bonded hydroxyl groups, confirming strong hydrophilicity. Peaks around 2922 cm^{-1} reflected alkyl C–H stretching, while the band at 2094 cm^{-1} suggested a nitrile group (C≡N). Carbonyl-related absorptions were detected between 1775 and 1700 cm^{-1}, and a prominent band near 1600 cm^{-1} indicated conjugated double bonds or aromatic structures, possibly from impurities or degradation products. Additional signals included CH_2/CH_3 bending vibrations (1438 cm^{-1}), C–N stretching or methyl bending (1356 cm^{-1}), and strong C–O stretching near 1021 cm^{-1}, which could also indicate siloxane linkages if silicates are present. Overall, the FTIR profile suggests a material rich in hydroxyl and ether functionalities, with minor contributions from carbonyl and aromatic groups, and traces of impurities likely from processing or environmental exposure.

CONCLUSION

The ethanolic extract of *Dodonaea Viscosa* demonstrated notable antibacterial activity against *Salmonella Typhi*, supporting its traditional medicinal use. Phytochemical analysis confirmed the presence of bioactive compounds such as alkaloids, flavonoids, glycosides, tannins, terpenoids and phenolic compounds. TLC revealed six distinct secondary metabolites. While UV-Vis and FTIR analyses indicated a comlex mixture of compounds with antimicrobial relevance. Antibacterial assays showed a dose-dependent effect with the highest activity of 100 mg/ml (zone of inhibition 12-13 mm), a MIC of 50 mg/ml and MBC of 100 mg/ml. these results suggest that *D. Viscosa* contains promising bioactive constituents warranting further isolation, characterization and In-Vivo evaluation for potential development into phytotherapeutic agent targeting antibiotic-resistant bacterial.

Recommendation

Further fractionation and isolation of the individual bioactive compounds using advanced chromatographic techniques (e.g., HPLC) is recommended.

Conduct in-vivo studies to evaluate the efficacy and safety of the extract in biological systems.

Explore the mechanism of action of the active constituents against specific bacterial strains.

Consider developing formulations for potential clinical application, particularly for topical or oral antimicrobial therapies.

Collaborate with pharmacologists and medicinal chemists to assess the potential for drug development based on the active principles of *D. viscosa*.

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