UNIJERPS
Unizik Journal of Educational Research and Policy Studies VOL.18 (3);
July-December, 2024 https://unijerps.org
2023 Impact Factor: **6.834;** 2022 ***Impact Factor***: 5.156; ***2021 Impact Factor: 5.641;***
2020 Impact Factor: 5.355. *Indexing and Impact Factor URL:* http://sjifactor.com/passport.php?id=21363

PHYTOCHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITIES OF ENDANGERED TREE SPECIES OF VITEX DONIANA SWEET AND DIOSPYROS MESPILIFORMIS HOCHST Ex A DC.

***[1]Ibrahim Abdullahi, [2]Isyaku Abubakar, [1]Bello Bello Musawa, [1]Mustapha Sani Muhammad and [1]Raphael Onyema**

[1]Department of Science Laboratory Technology, Federal Polytechnic Kaura-Namoda, P.M.B 1012 Zamfara State, Nigeria
[2]Biotechnology Advanced Research Centre, Sheda Science and Technology Complex, P.M.B 186, Garki, Abuja, Nigeria.
Corresponding author: ibrahimdauran491@gmail.com, +2348030612815,
https//:orcid.org/0009-0002-7642-7521

ABSTRACT

Antibacterial properties and phytochemical screening of two endangered tree species, Vitex doniana Sweet and Diospyros mespiliformis Hochst. ex A. DC. were examined. With voucher numbers BBM 156 and KN 88, the two plant samples were identified in the herbarium section of the biology department at Umaru Musa Yaradua University in Katsina after being gathered from Katsina State. Using the cold maceration method, the bioactive compounds found in both plant samples were extracted. The antibacterial activities were assessed using agar diffusion techniques, while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were investigated using tube diffusion techniques. Anthraquinones, flavonoids, terpenoids, alkanols, phenolics, saponins, and tannins were found in both plant samples, according to the study's findings. Antimicrobial zone of inhibition (mm) activity of the aqueous and ethanol extracts from the leaves of V. doniana revealed that at a 100 mg/ml dosage, S. aureus had a 20 mm zone of inhibition, while S. typhi and E. coli both had a high zone of inhibition (21 mm). For the stem back extracts, S. aureus exhibited the biggest inhibitory zone (23 mm), followed by E. coli (20 mm), S. typhi, and S. dysentriae (19 mm). Both aqueous and ethanolic extracts of V. doniana stem bark demonstrated minimum inhibitory concentrations (MIC) of 6.25 mg/ml and minimum bactericidal concentrations (MBC) of 12.5 and 25 mg/ml, respectively, against E. coli, K. pneumoniae, and S. dysentriae. S. aureus had MIC and MBC values of 12.5 mg/mL and 12.5 mg/ml, respectively. For Antimicrobial Zone of Inhibition (mm) activity of aqueous and ethanol extracts from leaves of D. mespiliformis, the result revealed that at a dosage of 100 mg/ml, S. dysentriae and S. aureus displayed the maximum zone of inhibition (22 mm), followed by S. typhi (20 mm) and K. pneumoniae (19 mm). On D. mespiliformis leaves, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous and ethanolic extracts were 6.25 mg/ml and 12.5 mg/ml. The results of the back extracts indicated that, at a dosage of 100 mg/ml, S. typhi and S. dysentriae had the highest inhibitory zone (21 mm), followed by E. coli (19 mm). On E. coli, K. pneumoniae, S. aureus, S. typhi, and S. dysentriae, MIC and MBC of aqueous and ethanolic extracts on D. mespiliformis stem bark were

6.25 mg/ml and 12.5 mg/ml respectively. Conclusively, both plants possess bioactive compounds that are effective against microbes. To identify the specific bioactive compounds responsible for these antimicrobial activities, it is recommended that further work should be done on these plants.

Keywords: Phytochemical, Antibacterial, Threatened species, Vitex doniana and Diospyros mespiliformis

Introduction

Plants have been vital to human survival since the beginning of time. They have given us medicine, food, fiber, and a variety of other beneficial products. Humans use a variety of plant parts on a daily basis, such as fruits, flowers, seeds, roots, stems, and leaves. Because practically every type of vegetation found in other African nations is found in large quantities in different parts of Nigeria. Nigeria is endowed with a diverse range of biodiversity in various geopolitical zones, making it one of the most bio-diverse countries on the continent (Namadina et al., 2020) Adeyemi and Ogundipe 2012; Ayodele et al., 2012 and Pelemo et al., 2011). Unfortunately, other unsustainable human activities are endangering these trees, and the population's attitude toward conservation may have improved. All levels of genetic resources will consequently unavoidably disappear. In a perfect world, everyone would have an inherent obligation to protect biodiversity (IUCN, 2010). This is untrue, though, as human activities that harm biodiversity and flora in general are happening more quickly. Almost 90% of Nigeria's forest has been cleared for human use (Kabiru, 2008; Santos and Ramos, 2018; Pelemo et al., (2011).

In addition to being found in grains, vegetables, fruits, and other plant products, phytochemicals are naturally occurring in the barks, leaves, vegetables, and roots of medicinal plants. They are known to have a protective effect against major chronic diseases in both infectious and host metabolic or genetic dysfunctional diseases (Lawal et al., 2022; Nayak et al., 2015) that are protected from a variety of illnesses by defense mechanisms (Esposito et al., 2016). Phytochemicals, which are plant-wide metabolites like sugars and lipids, make up the majority of transitional metabolic activities. Conversely, secondary metabolites are found in a small number of plants and have more diverse functions. Because of their therapeutic effects on humans, drugs such as digoxin (foxglove plant), quinine (cinchona plant), morphine and codeine (poppy plant), and inulin (dahlia plant) are made from secondary metabolites and pigments (Agunu et al., (2011). They are extensively used in scientific research, veterinary medicine, agriculture, human therapy, and a myriad of other fields. These secondary compounds include, among others, terpenoid, alkaloids, and phenolic compounds (Bosha et al., 2018). Plant-based drugs are widely available, affordable, safe, effective, and rarely have side effects. The best source of a variety of pharmaceuticals, according to World Health Organization (WHO) standards, would be medicinal plants. In

wealthy countries, traditional medicines are used by about 80% of the population.

Vitex doniana Sweet. and *Diospyros mespiliformis* Hochst ex A. DC. are one of the many plants that are utilized in Nigeria to treat a range of illnesses in both humans and animals (Ekom et al., 2018; Abdullahi et al., 2007; Ogbole and Ajaiyeoba, 2010; Aska and Kubmarawa, 2016), and the IUCN Red List has listed them as endangered species (Garba et al. 2021). Several scientific studies have shown that these plants contain a range of bioactive compounds (Ha et al., 2012; Haniadka et al., 2013).

The black plum, or *V. doniana*, is a tree plant that grows in savannah and opens woodland areas of tropical Africa, especially in northern, eastern, and western Nigeria. It is a member of the Lamiaceae family. It can reach a height of 20 meters and bears edible, sweet, plum-like fruits. When the fruits are fully ripe, their green color turns brown, and the pulp encircles a hard endocarp (Ushie et al., 2022; Lawal et al., 2021 and Okigbo, 2001). The Hausa call it Dinya, the Fulani call it Galbihi, the Yoruba call it Ori nla, and the Igbo call it Ucha koro. The extract of hot aqueous leaves *V. doniana* is used to treat a number of human conditions, including inflammatory diseases, diarrhea, dysentery, and rheumatic and stomach pains. Many people have used the roots to treat epilepsy and nausea. According to Lawal et al., (2022); Aiwonegbe et al., (2018); Jean et al., (2019) and Das et al., (2022) the tree stem back extract is used to treat indigestion, pain, disorders, and hypertension. *V. doniana* contains high concentrations of phytosterols, tocopherols (vitamin E), and the UV-absorbing triterpene cinnamates (Gholamine et al., 2019; Namadina, M.M et al., 2020). Badifu's 1989 study found that trees contain 5–15% unsaponifiable lipids and high antioxidant levels. These lipids include phytosterols like campesterol and triterpenes like lupeol, parkeol, butyrospermol, and cinnamic acid esters of α- and β-amyrin. Remarkably, quercetin and trans-cinnamic acid are also present in fruits at a concentration of around 0.4%. These polyphenolics include epicatechin, epicatechin gallate, gallocatechin, epigallocatechin, and epigallocatechin gallate (Frederick and Mani, 2016).

The African ebony, or *Diospyros mespiliformis*, is a tall, evergreen tree that can reach a height of 22 meters. It is a member of the Ebenaceae family and is also referred to as Kanya in Hausa and Igidudu in Yoruba. Its roots are densely rounded and buttressed. The tree is typically found in northern lowland forests and savannahs (Galo et al., 2022 and Bala, 2006). The tree's leaves are dark green, simple, and alternate, with tiny hairs on the underside of the older leaves. According to the National Research Council (NRC, 2008), *D. mespiliformis* is reportedly one of the most important genera of the Ebenaceae, whose species have been utilized in traditional medicinal systems for a long time. Phytochemicals and antibiotics are found in various plant parts, including the roots, leaves, fruits, and stem, and they have numerous therapeutic uses. In addition to aiding in childbirth, roots and stem bark are used as an anthelmintic and to treat infections like pneumonia, syphilis, leprosy, and dermatomycoses (Galo et al., 2022;

Mohamed and et al., 2009), whereas the leaves' infusion is used as a wound dressing agent, fever cure, and otitis treatment (NRC, 2008). Additionally, the leaves are used to treat skin infections, arthritis, and headaches. To treat gingivitis, toothaches, and wounds to prevent infection, the leaves and fruits are either chewed or applied as an infusion (John, 2008). Barks and roots are used to treat tumors and as psycho-pharmacological drugs (Galo et al., 2022; Mann et al., 2008). Similar studies on the pharmacological effects of *D. mespiliformis* by Falodun et al., (2009). According to phytochemical analyses of the plant's stem bark and seeds, limonoids were found (Daniel et al., 2024; Oyedeji et al., 2024). Additionally, saponins, alkaloids, phlobatanins, carbohydrates, and proteins were found (Santos and Ramos, 2018).

According to estimates from the World Health Organization, 90% of people globally use traditional medicine (Takin et al., 2013 and Korang et al., 2019). Resistance to antimicrobial drugs jeopardizes the effectiveness, prevention, and treatment of a growing number of diseases brought on by bacteria, parasites, viruses, and fungi (Tagliabue and Rappuoli, 2018; WHO, 2015; Nariya et al., 2011). Plant-based medications are essential for the prevention and treatment of diseases in both humans and animals. The phytochemical components of medicinal plants are linked to their pharmacological effects (Brown et al., 2016). The prevalence of drug resistance is drawing a lot of attention to the search for new and potent drugs made from plants. Novel phytochemicals that stop the growth of pathogenic organisms have been found in a variety of plant extracts (Vikrant et al., 2011; Paritala et al., 2015).

This research aims to perform phytochemical composition and evaluate the antibacterial activity of the vulnerable *V. doniana* and *D. mespiliformis* based on the medicinal relevance and conservation status of the tree species

Materials and Methods

Plant collection and identification

The samples of the leaves and bark of *V. doniana* and *D. mespiliformis* were gathered in Katsina State, Nigeria. The voucher specimens (BBM 156 and KN 88) and plant samples were identified in the herbarium section of the Department of Biology at Umaru Musa Yaradua University in Katsina. The fresh samples were sun-dried for seven days, then finely powdered and stored in plastic containers at room temperature (28°C) until needed.

Preparation of the extract

One hundred grams of powdered leaf and one hundred grams (100g) of bark were softened sequentially over the course of 72 hours (with periodic shaking) using the cold maceration method. Ethanol and distilled water, each containing one thousand milliliters (1000 ml), were utilized as extraction solvents. After passing through a Muslin cloth filter, the macerated samples were dried in a steam bath. To be used later, the dried extracts were weighed, placed in sterile sample vials, and refrigerated (Iyamabo, 1991).

Phytochemical Screening

Plant samples were extracted; their leaves and bark were examined using conventional

procedures for plant metabolites. Alkaloids, phenolics, tannins, saponins, flavonoids, terpenoids, steroids, and anthraquinones are some of these metabolites.

Screening for Alkaloids
In a steam bath, the extracts (0.5 g) were combined with 5 ml of 1% HCl and filtered while still hot. Wagner's reagent was added to 1 milliliter of the filtrate after the residue had been washed with distilled water. A positive alkaloid test resulted in a reddish-brown precipitate (Mojab et al., 2003).

Screening for Phenolics
After diluting the 0.2 g of chloroform extract with 50% ethanol, 3 drops of 10% ferric chloride were added. Phenols were present because a deep bluish-green solution appeared (Mojab et al., 2003).

Screening for Tannins
Three drops of 10% ferric chloride were added after the 0.2 g chloroform extract was diluted with 50% ethanol. The presence of phenols was suggested by the formation of a deep bluish-green solution (Mojab et al., 2003).

Screening Saponins
Extracts (2 ml) were cooked in a water bath with 20 ml of distilled water before being filtered. For a stable, long-lasting froth, ten (10) milliliters of the filtrate and five milliliters (5) of distilled water were combined and forcefully agitated. After adding three drops of olive oil to the mixture and giving it a good shake, the creation of an emulsion was watched for (Velavan, 2015).

Screening for Flavonoids
After dissolving the extracts (0.2 g) in 2 mL of water, they were filtered to remove flavonoids. Then, 2 mL of 10% aqueous sodium hydroxide was added. Flavonoids were represented by the yellow (Pandey and Tripathi, 2014).

Screening for Terpenoids
After combining 5 ml of extracts with 2 ml of chloroform, a layer was carefully formed by adding 3 ml of concentrated H2SO4. According to Chhetri et al., (2008) and Velavan (2015), the interface's reddish-brown coloration formed to indicate the presence of terpenoids.

Screening for Steroids
The extracts (5 ml) were combined with 2 ml of chloroform, and then a layer was carefully formed by adding 3 ml of concentrated H_2SO_4. The interface's reddish-brown coloration formed to indicate that terpenoids were present, which is a good sign (Chhetri et al., 2008; Velavan, 2015).

Screening for Anthraquinones
After shaking with 10 mL of benzene, extracts (0.5 g) were filtered. The filtrate was combined with a 10% aqueous ammonia solution, and the combination was agitated vigorously. According to Oloyede (2005) and Tadhani and Subhash (2006), the presence of anthraquinones is indicated by the emergence of pink and red color in the aqueous state.

Determination of antimicrobial activities
The Agar-Well Diffusion technique, as outlined by Lino and Deogracious (2006), was used for this. In order to evaluate the

plant extract's antibacterial properties, 0.8 g of each extract was dissolved in dimethyl sulfoxide (DMSO), yielding various extract concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml, in that order.

A standard inoculum of 1.5x108 cells, which matched 0.5 McFarland standards, was streaked on the surface of sterile Muller Hinton agar plates in triplicate. 0.1 ml of each plant extract was applied to a hole made in the Muller Hinton agar plates using a sterile 6 mm cork borer. For a whole day, the plates were incubated at 37^0C. By measuring inhibition zones in millimeters, the antibacterial activity was ascertained (Omar et al., 2000). The bactericidal activity of synthetic antibiotics was also tested on standardized discs, and inhibition zones were identified.

Determination of the minimum inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) was calculated using the tube dilution method (Adesokan et al., 2007). To get different concentrations, the plant extracts were serially diluted from the 100 mg/ml solutions. 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml were the concentrations. After combining multiple dilutions of the extract with Muller-Hinton broth, 0.1 ml of each standardized solution was added to a test tube containing a separate concentration of the test organisms. A positive control set of test tubes contained both test organisms and Mueller-Hinton broth, while a negative control set of test tubes had just Mueller-Hinton soup. The test tubes and the controls were then incubated at 37 °C for a whole day. After the incubation period, each tube exhibits either growth or no growth. To find out if the bacterial growth was stopped, a loop full from each tube was further sub-cultured on nutritional agar. The development of bacteria on solid medium suggested that the bacteria could not be inhibited by a specific dose of extract. The amount of extract, measured in grams per milliliter, to which the organism is sensitive, was indicated by the lowest concentration of extract that showed no growth.

Determination of Minimum Bactericidal Concentration (MBC)

One milliliter (1 ml) of broth culture was taken out of the tubes used to calculate the minimum inhibitory concentration (MIC) and subculturing it onto new solid nutrient agar plates allowed for the calculation of the minimum bactericidal concentration. For 24 hours, the plates were incubated at 37 ^{0}C. The MBC was defined by Adesokan et al., (2007) as the concentration that did not exhibit any growth following incubation.

Presentation of Results

By removing 1 ml of the broth culture from the tubes used to estimate the minimum inhibitory concentration (MIC) and subculturing it into new solid nutrient agar plates, the minimum bactericidal concentration was ascertained. For twenty-four hours, the plates were incubated at 37^0C. According to Adesokan et al., (2007), the concentration that showed no signsof development during incubation was considered the MBC as presented in table 1.

Table 1: Phytochemical screening of *Vitex doniana* and *Diospyros mespiliformis*

Phytochemical constituents	*V. doniana*		*D. mespiliformis*	
	Aqueous	**Ethanol**	**Aqueous**	**Ethanol**
Alkaloids	+	+	+	+
Phenolics	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	-	-
Flavonoids	+	+	-	-
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Anthraquinones	-	-	+	+

- = negative, + = positive

Table 2: Antimicrobial Zone of Inhibition (mm) Activity of Aqueous and Ethanol Extract from leaves of *V. doniana*

Bacterial isolates	Concentration (mg/ml)								Control	
	Extracts	100	50	25	12.5	6.25	MIC	MBC	Cipro 10 mg	Am 10 mg
Escherichia coli	Aqueous	21	18	16	13	10	6.25	12.25	20	26
	Ethanolic	19	16	13	11	9	6.25	12.25	21	30
Staphyloccocus aureus	Aqueous	18	16	15	13	11	6.25	12.25	23	31
	Ethanolic	20	18	15	12	11	6.25	12.25	27	31
Salmonella typhi	Aqueous	21	19	17	14	11	6.25	12.25	32	25
	Ethanolic	15	13	11	8	5	6.25	12.25	30	28
Shigella dysentriae	Aqueous	17	15	13	11	9	6.25	12.25	20	21
	Ethanolic	16	14	11	10	7	6.25	12.25	32	31
Klebsiella pneumonia	Aqueous	17	15	13	11	8	6.25	12.25	25	21
	Ethanolic	18	15	11	9	6	6.25	12.25	26	29

Cipro = Ciprofloxacin, Am= Ampicillin, MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration

Table 2 results demonstrated the antibacterial activity of various extract concentrations on isolates of *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Shigella dysentriae, and Klebsiella pneumoniae* for both ethanolic and aqueous extracts of *V. doniana*. The outcomes demonstrated that the microorganism's growth inhibition zone increased as extract concentration rose. As usual, medications, ampicillin and ciprofloxacin, both broad-spectrum antibiotics, were administered against the cultured bacteria. *S. typhi* and *E. coli* both had a high zone of inhibition (21 mm), according to the results (Table 2). *S. aureus* came in second with a zone of inhibition of 20 mm at a 100 mg/ml dose. On *V. doniana* leaves, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous and ethanolic extracts were 6.25 mg/ml and 12.5 mg/ml on *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella dysentriae*, and *Klebsiella pneumoniae*, *respectively.*

Table 3: Antimicrobial Zone of Inhibition (mm) Activity of Aqueous and Ethanol Extract from the stem bark of *V. doniana*

Bacterial isolates	Concentration (mg/ml)							Control	
	Extracts	100	50	25	12.5	6.25	MIC	MBC	Cipro 10 mg	Am 10 mg
Escherichia coli	Aqueous	17	15	13	10	8	6.25	6.25	24	21
	Ethanolic	20	18	15	13	11	6.25	25	31	25
Staphyloccocus aureus	Aqueous	23	20	18	16	14	12.5	12.5	20	22
	Ethanolic	18	16	14	12	10	12.5	12.5	23	28
Salmonella typhi	Aqueous	19	16	13	11	8	6.25	6.25	32	24
	Ethanolic	17	15	12	10	9	6.25	6.25	22	26
Shigella dysentriae	Aqueous	19	17	14	12	10	6.25	6.25	21	22
	Ethanolic	15	13	12	10	8	6.25	6.25	20	24
Klebsiella pneumonia	Aqueous	16	14	12	10	9	6.25	6.25	20	21
	Ethanolic	15	14	12	10	8	6.25	6.25	30	28

Cipro = Ciprofloxacin, Am= Ampicillin, MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration

At a concentration of 100 mg/ml, the antibacterial activity result (Table 3) revealed that at the greatest dose, *S. aureus* had the largest inhibition zone (23 mm), followed by *E. coli* (20 mm), *S. typhi*, and *S. dysentriae* (19 mm). On *V. doniana* stem bark, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous and ethanolic extracts were 6.25 mg/ml and 12.5 and 25 mg/ml, respectively, on *E. coli*, *K. pneumoniae*, and *S. dysentriae*. MIC and MBC values for *S. aureus* were 12.5 mg/mL and 12.5 mg/mL, respectively.

Table 4: Antimicrobial Zone of Inhibition (mm) Activity of Aqueous and Ethanol Extract from leaves of *D. mespiliformis*

Bacterial isolates	Concentration (mg/ml)								Control	
	Extracts	100	50	25	12.5	6.25	MIC	MBC	Cipro 10 mg	Am 10 mg
Escherichia coli	Aqueous	18	16	15	14	11	6.25	12.25	28	22
	Ethanolic	17	15	13	11	9	6.25	12.25	29	26
Staphyloccocus aureus	Aqueous	22	19	16	12	10	12.25	12.25	22	26
	Ethanolic	19	18	16	14	11	12.25	12.25	20	19
Salmonella typhi	Aqueous	20	17	14	12	10	6.25	12.25	22	24
	Ethanolic	18	17	15	13	10	6.25	12.25	30	31
Shigella dysentriae	Aqueous	17	15	13	10	9	6.25	12.25	22	20
	Ethanolic	22	18	16	14	11	6.25	12.25	27	21
Klebsiella pneumonia	Aqueous	18	15	12	10	9	12.25	12.25	20	24
	Ethanolic	19	16	13	10	8	12.25	12.25	22	21

Cipro = Ciprofloxacin, Am= Ampicillin, MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration

At a dosage of 100 mg/ml, the antibacterial activity result showed that *S. dysentriae* and *S. aureus* displayed the maximum zone of inhibition (22 mm), followed by *S. typhi* (20 mm) and *K. pneumoniae* (19 mm). On *D. mespiliformis* leaves, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous and ethanolic extracts were 6.25 mg/ml and 12.5 mg/ml, respectively. *S. typhi*, *E. coli*, and *S. dysentriae* were shown to have MICs and MBCs of 12.5 mg/mL and 12.5 mg/mL, respectively, for *Staphylococcus aureus* and *K. pneumoniaea*.

Table 5: Antimicrobial Zone of Inhibition (mm) Activity of Aqueous and Ethanol Extract from the stem bark of *D. mespiliformis*

Bacterial isolates	Concentration (mg/ml)								Control	
	Extracts	100	50	25	12.5	6.25	MIC	MBC	Cipro 10 mg	Am 10 mg
Escherichia coli	Aqueous	19	17	14	12	10	6.25	12.5	20	22
	Ethanolic	19	18	16	14	12	6.25	12.5	31	24
Staphyloccocus aureus	Aqueous	15	13	12	10	9	6.25	12.5	25	26
	Ethanolic	16	14	12	10	8	6.25	12.5	21	30
Salmonella typhi	Aqueous	21	19	17	14	11	6.25	12.5	32	30
	Ethanolic	18	17	15	12	10	6.25	12.5	22	20
Shigella dysentriae	Aqueous	21	18	15	12	10	6.25	12.5	25	28
	Ethanolic	17	15	13	10	8	6.25	12.5	23	21
Klebsiella pneumonia	Aqueous	16	14	12	10	9	12.5	12.5	20	30
	Ethanolic	17	15	14	12	10	12.5	12.5	32	34

Table 5 showed *S. typhi* and *S. dysentriae* had the maximum inhibition zone (21 mm), followed by *E. coli* (19 mm) at a dose of 100 mg/ml, according to the antibacterial activity result (Table 5). On *E. coli*, *K. pneumoniae*, *S. aureus*, *S. typhi*, and *S. dysentriae*, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous and ethanolic extracts on *D. mespiliformis* stem bark were 6.25 mg/ml and 12.5 mg/ml, respectively. In contrast, 12.5 mg/mL and 12.5 mg/mL were shown to be the MIC and MBC of *K. pneumoniaea.*

Discussion of Findings

On a global scale, plants are widely recognized as a vital resource and an integral component of Earth's biological diversity. Because they are used by people worldwide for food, medicine, fuel, clothing, and shelter, tens of thousands of wild plants are significant both economically and culturally. Additionally, plants are essential to the stability of ecosystems and the preservation of the Earth's environmental balance. The animal and insect life of the world can also be found there. Many plant species are currently in danger of becoming extinct as a result of pollution, climate change, overexploitation, invasive alien species, industrialization, deforestation, and changes to their natural habitats. The loss of significant biodiversity is one of the biggest obstacles the international community faces in its efforts to stop the extinction of plant diversity. Two of the most vulnerable tree species on the IUCN Red List are *Vitex doniana* and *Diospyros mespiliformis* (Garba et al., 2021). The current study indicates that the bioactive compounds present in *V. Doniana* and *D. mespiliformis* are essential for the treatment of human and animal diseases. Using phytochemical screening, the *V. doniana and D. mespiliformis* was found to contain alkaloids, phenolics, tannins, saponins, flavonoids, and steroids; however, anthraquinones were not present in either of the aqueous or ethanolic extracts of the plant; in a similar vein, neither of the aqueous nor ethanolic extracts of *D. mespiliformis* contained saponins or flavonoids. This aligns with the study carried out by Aiwonegbe et al., (2018), Moriasi et al., (2024); Alagbe and Oluwafemi (2019); Aumeeruddy and Mahomoodally (2021). *V. doniana and D. mespiliformis* have been shown to have secondary metabolites, which are typically present in plant components, according to reports from several researchers (Truong et al., 2019; Markossian et al., 2021; Galo et al., 2022; Breslin, 2017). Numerous studies have documented the presence of secondary metabolites that have significant inhibitory effects on many species (Alagbe and Oluwafemi 2019). The antibacterial activity of *V. doniana and D. mespiliformis* may be attributed to these secondary metabolites, which elicit their antimicrobial effects via distinct processes.

The antimicrobial zone of inhibition data showed that the aqueous extract was more active than the ethanolic extract. The level of activity varied according to the extracts, the type of bacterial isolate, and the dosage. The vulnerable organisms produced larger inhibition zones than the resistant species. These antibacterial qualities might be related to the bioactive components in the plant

extracts. Bioactive compounds including flavonoids, phenolics, tannins, alkaloids, saponins, and steroids were present in the plant extracts. These bioactive compounds did, however, have antibacterial properties. Another explanation could be the solubility of the solvents; water is a more polar solvent and thus dissolves more secondary metabolites. The results are consistent with research by Lawal et al., (2022) and Olarinke (2017), in addition to Namadina et al., (2020), which showed that aqueous extracts were more active than extracts using ethanol, methanol, and chloroform. Mohammed et al., (2012) conducted a similar study at the Aminu Kano Teaching Hospital in Kano and discovered that the most effective treatment for *S. aureus* isolated from wound samples was an extract of *Alchornea cordifolia*. The minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) values of the aqueous and ethanolic extracts were considerably lower, ranging from 6–25 to 12–5 mg/ml.

This implies that MIC and MCB measurements are all that were needed to measure bacterial activity (Namadina et al., 2020). The MIC and MCB values showed that the aqueous extract was more effective against the test organisms than the ethanolic extract. The results showed that the common antibiotics used as a positive control were positively impacted by the plant extracts (Adewunmi et al., 2001 and Abdelgaleil et al., 2004). It is commonly known that this plant's antibacterial qualities are due to the phytochemicals it contains. The findings of this investigation align with those of Namadina et al., (2020), demonstrating that *V. doniana's* root and stem bark worked better against *E. coli* isolates from patients who visited the general outpatient clinic (GOP) at the Murtala Muhammad Specialist Hospital in Kano State with upper and lower respiratory tract infections. Additionally, this is consistent with Osumah et al., (2012) findings, who demonstrated that extracts from the stem bark and roots of *A. cordifolia* as well as *A. cordifolia* fractions were more effective than *S. aureus* isolated from wound and fecal samples at Ahmadu Bello University Teaching Hospital Zaria. The composition of the organisms' cell walls may have an impact on differences in the isolates' susceptibilities to the plant extracts.

Conclusion

The antibacterial and phytochemical properties of two threatened tree species, *V. doniana* and *D. mespiliformis* were investigated. The results of the study gave the traditional uses of these plants scientific validation. The findings showed that both trees contained bioactive components phenolics, alkaloids, tannins, terpenoids, and steroids. The aqueous and ethanolic extracts of *V. doniana* included flavonoids and saponins, but neither the aqueous extract of *D. mespiliformis* nor its anthraquinones were present. It has also been established that the leaves and stem bark of *V. doniana and D. mespiliformis* have broad-spectrum antibacterial action against *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Shigella dysentriae,* and *Klebsiella pneumoniae.*

REFERENCES

Abdullahi, M., Amupitan, J.O., Oyewale, A.O., Okogun, J.I., Kolo, I., (2007). An ethnobotanical survey of indigenous flora for treating tuberculosis and other respiratory diseases in Niger State, Nigeria. Journal of Phytomedicine and Therapeutics; 2007; 12, 1–21.

Adesokan, A. A., Akanji, M. A. and Yakubu, M. T. (2007). Antibacterial potentials of aqueous extract of *Enantia chlorantha* stem bark. African Journal of Biotechnology, 6(22). 112-119.

Adeyemi, T.O. and Ogundipe, O.T. (2012). Biodiversity of Sapindaceae in West Africa: A checklist: International Journal of Biodiversity and Conservation 4 (10):358-363.

Agunu, A. Ahmadu, A.A., Afolabi, S.O., Yaro, A.U., Ehinmidu, J.O. and Mohammed, Z. (2011). "Evaluation of the antibacterial and antidiarrhoeal activities of *Heeria insignis*." *Indian Journal of Pharmaceutical Sciences*, 73(3): 328–332.

Aiwonegbe, A. E., Iyasele, J. U. and Izevbuwa, N. O. (2018). Proximate composition, Phytochemical and Antimicrobial Screening of the Methanol and Acetone extracts of Vitex doniana fruit pulp. Ife journal of Science. 20 (2) 317-323.

Alagbe, J.O and Oluwafemi, R.A. (2019). Growth performance of weaner rabbits fed Noni (Morinda citrifolia) and Moringa olifera leaf mixture as partial replacement of soya bean meal. International journal of Advanced Biological and Biomedical Research. 7(2):185-195.

Arunkumar, S. and Muthuselvam. (2009). Analysis of phytochemical constituents and antimicrobial activities of aloe vera L. against clinical pathogens. *World J. Agril. Sci*, 5(5): 572-576.

Aska, A.S., Kubmarawa, D., Preliminary Phytochemical Screening of Some Indigenous Medicinal Plants Used In the Treatment of Tuberculosis in Bauchi State, Nigeria. IOSR Journal of Applied Chemistry, 2016; 9 (4), 48- 52

Aumeeruddy, M. Z., and Mahomoodally, M. F. (2021). Global documentation of traditionally used medicinal plants in cancer management: a systematic review. South Afr. J. Bot. 138, 424–494. doi:10.1016/j.sajb.2021.01.006

Ayodele AE & Yang Y (2012) Diversity and distribution of vascular plants in Nigeria. Published by Qingdao Publishing House, Qingdao, China, 350 p

Badifu GIO. Lipid composition of Nigerian *Butyrospermum paradoxum* kernel. Journal of Food Composition and Analysis. 1989; 2:238-244

Bala SA. Common ethnomedicinal plants of the semiarid regions of West Africa: Their description and phytochemicals. Triumph Publishing Company. 2006; 1: 1-266.

Bosha, J. A., Anaga, A. O. and Asuzu, I. U. (2018). Chemical Composition of *Newbouldia Laevis* (P. Bauv) Used in the Treatment of Diabetes Mellitus in Nigeria. Journal of Nutrition, 1: 105

Breslin A (2017) "The Chemical Composition of Green Plants". Sciencing, Leaf Group Ltd.

Brown, L., C. Langelier, M. J. Reid, R. L. Rutishauser and L. Strnad (2016). "Antimicrobial

Chhetri HP, Yogol NS, Sherchan J, Anupa KC, Mansoor S, Thapa P. Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal. Kathmandu University Journal of Science, Engineering and Technology. 2008; 1:49-54.

Daniel J. L., Vandi P., Chessed, G., Umar, M. and Yusuf, A. I. (2024). Phytochemical Analysis of Medicinal Plants Used in the Treatment of Malaria Infection in Billiri and Funakaye Local Government Areas of Gombe State

Das, N., Salgueiro, A. C. F., Choudhury, D. R., Mandal, S. K., Logesh, R., Hassan, M. M., et al. (2022). Traditional uses, phytochemistry, and pharmacology of genus Vitex (Lamiaceae). Phytotherapy Res. 36 (2), 571–671. doi:10.1002/ptr.7330

Ekom, S. E. and Tamokou, J. D. (2018). Methanol Leaves Extract of *Psidium guajava* Linn. Exhibited Antibacterial and Wound Healing Activities. *International Journal of Current Microbiology and Applied Science*, 7(7): 4008-4023.

EXTRACTS OF *VITEX DONIANA. Scientia Africana, Vol. 21 (No. 1), April, 2022. Pp 149-158*

Falodun, A., Poh, C.F., Adelusi, S.A., Emmanuel, O., (2009): Phytochemical and anti-inflammatory evaluation of *D. mespiliformis* stem bark extract. Int. J. PharmTech. Res., 1(4): 1061-1064.

Frederick, N. and Mani, J.V. (2016). Phytochemical screening and In-Vitro Antioxidant activity of different solvent extracts of *Musa rasthali* fruit. *International Journal of Chemical and Physical Sciences*, 5(3):6-9.

Galo Yahaya Sara, Luka Yelwa Barde , Mohammed Mohammed and Mohammed Kabiru (2022) Elemental and phytochemical analysis of *Diospyros mespiliformis* leaves and stem bark in Nangere local government area of Yobe state, Nigeria

Garba, A. Salami, K. D. and Akanbi, W. B. (2021) Assessment of Endangered Economic Tree Species And Conservation Techniques In Jigawa State, Nigeria. *FUDMA Journal of Agriculture and Agricultural Technology ISSN: 2504-9496 Vol. 7 No. 2, ecember 2021: Pp .116-123* DOI: https://doi.org/10.33003/jaat.2021.0702.056

Gholamine, G., Houshmand, A., Hosseinzadeh, M., Kalantar, S., Mehrzadi, M. and Goudarzi, L. (2019). Gallic acid ameliorated sodium arsenite-induced renal and hepatic toxicity in rats. *Drug Chemistry and Toxicology*, 25: 1–12.

Ha, S.K., Moon, E., Ju, M.S., Kim, D.H., Ryu, J.H., Oh, M.S., Kim, S.Y., Shogaol, A Ginger Product, Modulates Neuroinflammation: A New Approach to Neuroprotection. Neuropharmacology, 2012; 63:211-23.

Hamdillah A, Isnansetyo A, Istiqomah I, Puspita ID, Handayani DP, Kaneko T. 2019. Antibacterial activity of coastal plants and marine sponges from Kei Island Indonesia against bacterial fish

pathogens. Pharmacognosy J 11: 1-6. DOI: 10.5530/pj.2019.11.130.

Haniadka, R., Saxena, A., Shivashankara, A.R., Fayad, R., Palatty, P.L., Nazreth, N., Francis, A., Arora, R., Baliga, M.S., Ginger Protects the Liver against the Toxic Effects of Xenobiotic Compounds: Preclinical Observations. Journal of Nutrition and Food Sciences 2013; 3:226

IUCN (2010). IUCN, Gland bulletin. Retrieved from: http://www.iucn.org

Iyamabo PA (1991). Thesis on comparative antimicrobial activity of crude extract of *Terminalia macroptae* with phenol chlorhexidine and gentamycin. Pharmacognosy Journal 1(1):12.

Jean, B. M., Nag-Tiero, M. R., Hermann, O. Y., and Germaine, N. O. (2019). Traditional uses, phytochemistry and pharmacology review of 2 Vitex: diversifolia and doniana. Int. J. Res. Dev. Pharm. and Life Sci. 8 (2), 29–36. doi:10.21276/ijrdpl.2278-0238.2019.8(2).29-36

John T. P (2008). Segmented Double-Stranded RNA Viruses: Structure and Molecular Biology. Bethesda, MD 20892-8026, USA: Caster Academic Press. ISBN 978–1–90 4455–21–9 DOI: https://doi.org/10.21775/9781910190753

Kabiru, Y. (2008). Nigeria's Forest to disappear by 2020. African Conservation Foundation. Network news report.

Korang J., Opuni-Frimpong E., Ofori E. and Owusu J. (2019) Comparative analysis of the phytochemical constituents and antimicrobial activities of four species of African mahogany

Lawal, H. Z., Olatunji, K. and Abdulazeez, A. (2022) Comparative Study On The Proximate, Mineral, Phytochemicals And Antimicrobial Analysis OF Daniellia oliveri, Leptadenia hastata AND Vitex doniana LEAVES. *Special Conference Edition, April, 2022*

Lino A, Deogracious O (2006). The in-vitro antibacterial activity of *Annona senegalensis*, *Securidaccalongi pendiculata* and *Steganotaenia araliacea* - Ugandan Medicinal Plants. African Health Sciences 6(1):31-35

M.O. Oyedeji Amusa, R.D. Stewart, M. van der Bank, B.-E. van Wyk, A Taxonomic Review of South African Indigenous Meliaceae Using Molec-ular Systematics and Anatomical Data, Diversity 16 (2024) 113.

Mann A, Yahaya Y, Banso A, John F. Phytochem and antimicrobial activity of *Terminalia avicennioides* extracts against some bacteria pathogens associated with patients suffering from complicated respiratory tract diseases. J. Med. Plants Res. 2008; 2(5): 94-97.

Markossian, S., Grossman, A., Brimacombe Michelle Arkin, K., Auld, D., Austin Jonathan Baell, C. P., Chung, T. D., et al. (2021). Assay guidance manual. Available at: https://ncats.nih.gov/expertise/preclinical/agm, 351–358.

Mohamed IE, El Nur EE, Choudhary MI, Khan SN. Bioactive natural products from two Sudanese medicinal plants *Diospyros mespiliformis* and *Croton zambesicus*. Rec. Nat. Prod. 2009; 3: 198-203.

Mohammed, A., Adeshina, G. O., Ibrahim, Y. K. E. (2012). Incidence of wound

infections in a teaching hospital in Kano and susceptibility of the bacteria isolates to *Alchornea cordifolia* leaf extracts. M.sc Research Dissertation (Unpublished).Department of Pharmaceutics/ Pharmaceutical Microbiology Library. Ahmadu Bello University, Zaria.

Mojab F, Kamalinejad M, Ghaderi N, Vahidipour HR. Phytochemical screening of some species of Iranian plants. Iranian Journal of Pharmaceutical Research. 2003; 2:77-82.

Moriasi G, Ngugi M, Mwitari P and Omwenga G (2024) In vitro anti-prostate cancer efficacy and phytochemical composition of the dichloromethane and ethyl acetate leaf extracts of Vitex doniana (sweet).

Namadina, M.M, Idris, A. M, Sunusi, U, Abdulrazak, M.H, Musa, F. M, Olaogun, O.N, Aminu, M. A, Yakubu, N, Sani, M.H, Ali, Z.A, Nuhu, Y, Makin, T.Y, Gwarzo, A.M (2020). Phytochemical and Antibacterial Activities of *V. doniana* Stem Bark and Root Extracts against Some Clinical Isolates of Respiratory Tract Infections.

Namadina, M.M, Idris, A. M, Sunusi, U, Abdulrazak, M.H, Musa, F. M, Olaogun, O.N, Aminu, M. A, Yakubu, N, Sani, M.H, Ali, Z.A, Nuhu, Y, Makin, T.Y, Gwarzo, A.M (2020). Phytochemical and Antibacterial Activities of *Vitex donianna* Stem Bark and Root Extracts against Some Clinical Isolates of Respiratory Tract Infections.

Nariya, P. B., Bhalodia, N. R., Shukla, V. J., Acharya and R. N. (2011). Antimicrobial and antifungal activities of Cordia dichotoma (Forster F.) bark extracts, Journal of Research in Ayurveda, 32 (4): 585 – 589.

National Research Council. 2008. *Lost Crops of Africa: Volume III: Fruits*. Washington, DC: The National Academies Press. https://doi.org/10.17226/11879.

Ogbole, O.O., Ajaiyeoba, E.O., Traditional management of tuberculosis in Ogun state of Nigeria: the practice and ethnobotanical survey. African Journal of Traditional, Complementary and Alternative Medicines, 2010; 7(1):79–84.

Olarinke Victoria Adeniyi, Flora E. Olaifa, B. O. Emikpe and S. T. Ogunbanwo (2017) Phytochemical Components and Antibacterial Activity of *Tamarindus indica* Linn. Extracts against Some Pathogens

Oloyede OI. Chemical profile of unripe pulp of *Carica papaya*. Pakistan Journal of Nutrition. 2005; 4:379-381.

Omar S, Lemonnier B, Jones N, Ficker C, Smith ML, Neema C, et al. Antimicrobial activity of extracts of eastern North American hardwood trees and relation to traditional medicines. J Ethnopharmacol 2000;73:161-70.

Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry. 2014; 2:115-119.

Paritala, V., Chiruvella, K. K., Thammineni, C., Ghanta, R. G. and Mohammed, A.

(2015). Revista Brasileira de Farmacognosia, 25: 61 – 83.

Pelemo, O.J., Akintola, B.A., Temowo, O.O., Akande, E.O. and Akoun, M. (2011). Effects of Landscape Change on Biodiversity in Nigeria: Remote Sensing and GIS Approach. Continental Journal of Environmental Design and Management 1 (2): 22 – 29.

Santos L, Ramos F. 2018. Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. Intl J Antimicrob Agents 52: 135-143. DOI: 10.1016/j.ijantimicag.2018.03.010.

Santos L, Ramos F. 2018. Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. Intl J Antimicrob Agents 52: 135-143. DOI: 10.1016/j.ijantimicag.2018.03.010.

Tadhani M, Subhash R. Preliminary studies on Stevia rebaudiana leaves: Proximal composition, mineral analysis and phytochemical screening. Journal of Medical Sciences. 2006; 6:321-326

Tagliabue, A. and R. Rappuoli (2018). "Changing priorities in vaccinology: antibiotic resistance

Takin, M. C., Attindenou, S., Sezan, A., Attakpa, S. E. and Baba-Moussa, L. (2013). Bioactivity, therapeutic utility and toxicological risks of *Diospyros mespiliformis*, Indian Journal Pharmacy and Biological Research, 1 (4): 122 - 129

Truong, D. H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., and Nguyen, H. C. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of severinia buxifolia. J. Food Qual. 2019, 1–9. doi:10.1155/2019/8178294Available at: https://www.hindawi.com/ journals/jfq/2019/8178294/.

Ushie, O. A., Longbap, B. D., Azuaga, T.I., Iyen, S. I., Ugwuja, D. I and Ijoko, R. F. (2022) PHYTOCHEMICAL SCREENING AND PROXIMATE ANALYSIS OF THE LEAF

Velavan, S. (2015). Phytochemical Techniques - A Review. *World Journal of Science and Research, 2*(1), 80-91.

Vikrant, A. and Arya, M. L. (2011). A review on Anti-Inflammatory plant barks. International Journal of PharmTech Research, 3 (2): 899 - 908. WHO, (2015) Fact sheet N°194 http://www.who.int/mediacentre/factsheets/fs19 4/en/

World Health Organization. (2015). Traditional Medicine. http//www.who.int/mediacentre/factshee t/fsl34/en/print/html