

Phytochemistry and Biological Activity of Stem Bark and Leaf Extract of *Vitex dioniana* and *Detarium microcarpum* Plants

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Abstract: Medicinal plants remain one of important sources of bioactive compounds for the discovery of therapeutic agents. This study investigated the phytochemical composition, antioxidant, antimicrobial, and anti-inflammatory activities of *Vitex doniana* and *Detarium microcarpum* extracts (stem bark and leaf). Plant sample was extracted with methanol using soxhalet extractor. Phytochemical screening was carried out using standard method. Antioxidant activities was assessed using DPPH radical scavenging. Antimicrobial activities was evaluated using the agar wall diffusion method and anti-inflammatory activities was carried out using inhibition of albumin denaturation (in vitro). The study showed the presence of alkaloids, flavonoids, tannins, steroids, and glycosides on both the plant extracts. The antioxidant activity of *Vitex donian* extract exhibited percentage inhibition ranged from 79.9% at 2ug/ml to a peak of 93.2% at 60ug/ml, and the leaf extract robust antioxidant effects with inhibition increasing from 59.5% at 20ug/ml to a maximum of 91.3% at 80ug/ml, while *Detarium microcarpum* stem extract displayed a gradual concentration from 15.8% at 20ug/ml to 85.5% at 200ug/ml and the leaf extract ranging from 61.4% at 20ug/ml to 95.6% at 60ug/ml. Antimicrobial sensitivity of *Detarium microcarpum* leaf extract showed *Staphylococcus aureus* concentration rose from 25ug/ml to 100ug/ml (16mm, 11mm, 13mm 9mm, 7mm), *Staphylococcus epidermidis* ranges from (11mm, 9mm, 6mm, 6mm), *Escherichia coli* (9mm, 8mm, 7mm, 6mm), *Salmonella typhi* (18mm, 13mm, 12mm, 10mm), the fungal showed *Candida albicans* (14mm, 11mm, 9mm, 8mm) and *Aspergillus niger* (17mm, 12mm, 8mm, 6mm). the stem bark extract showed *Staphylococcus aureus* ranges from (11mm, 6mm, 6mm, 6mm), *Staphylococcus epidermidis* (12mm, 9mm, 8mm, 7mm), *E. Coli* (7mm, 6mm, 6mm, 6mm), *Salmonella typhi* (13mm, 11mm, 9mm, 7mm), *Candida albicans* (11mm 9mm, 6mm, 6mm), *Aspergillus niger* (14mm, 11mm, 9mm, 8mm). And for *Vitex doniana* leaf extract showed *Staphylococcus aureus* (19mm, 16mm, 13mm 9mm), *Staphylococcus epidermidis* (14mm, 11mm, 9mm, 7mm), *Escherichia coli* (13mm, 9mm, 8mm, 7mm), *Salmonella*(12mm, 9mm, 8mm, 6mm), *Candida* (16mm, 13mm, 9mm, 8mm), *Aspergillus niger* (8mm, 6mm, 6mm, 6mm) and the stem extract are *Staphylococcus aureus* (12mm, 9mm, 8mm, 7mm), *Staphylococcus epidermidis* (13mm, 11mm, 6mm, 6mm), *Escherichia coli* (14mm,13mm, 10mm, 8mm), *Salmonella typhi* (11mm, 8mm, 7mm, 6mm), *Candida albicans* (15mm, 13mm, 11mm, 9mm) and *Aspergillus niger* (16mm, 12mm, 9mm, 6mm). In vitro anti-inflammatory activity of *Vitex doniana* stem extract showed absorbance value ranges from 10 to 500ug/ml which rise in inhibition from 14.34% to 78.65%, the leaf extract range from 5.43% at lower concentration to 82.13% at 500ug/ml and the stem extract of *Deterium microcarpum* inhibited from 6.59% at 10ug/ml to 53.72 at 500ug/ml, the leaf extract inhibited from 29.84% at lowest concentration to 61.24% at 500%. The findings suggest that *V. doniana* and *D. microcarpum* contain bioactive constituents with potential pharmaceutical applications.

Keywords: Antioxidant, Antimicrobial, Glycoside, Plant, Phytochemicals *Vitex doniana*.

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I. INTRODUCTION

The term traditional medicine refers to different medicinal practices around the globe and it is also known as complementary or alternative medicine in some areas (WHO, 2000). Plants are an abundant source of bioactive compounds that could be considered as leads for novel drugs that will be more potent and less toxic. Even though some plant extracts are used as medicines themselves, identification of active compounds and the ways they work will be important for their successful utilization in drug development process (Ahad et al., 2021; Rahayu et al., 2020; Darsini et al., 2015; Kumar et al., 2005). Pain is a multifaceted disease which includes sensory, emotional, cognitive, and social elements and is characterized as being either acute, chronic, nociceptive or neuropathic (Williams Craig, 2016; Leyva et al., 2019). The common treatments include nonsteroidal anti-inflammatory drugs and corticosteroids. They are effective but may cause side effects, which include gastrointestinal and renal toxicity, immunosuppression, osteoporosis and hyperglycemia if used for a long period (Payne, 2000; Wong, 2019; Manson et al., 2009). Inflammation is a physiological reaction in response to injury but its excessive or prolonged presence contributes to different diseases such as rheumatoid arthritis, glomerulonephritis, hepatitis and vasculitis. Inflammation is mediated by different inflammatory mediators such as prostaglandins, leukotrienes, and cytokines. Oxidative stress resulting from overproduction of ROS results in damages to proteins, lipids, nucleic acids and enzymes and worsens the disease (Dinarello et al., 2010; Sies et al., 2017). Due to the shortcomings of the current treatment options, there is an increasing interest in the use of natural therapies. There is an association between free radicals and different diseases, including cancer, diabetes, obesity, inflammation and degenerative diseases. Antioxidants will play a role in alleviating oxidative stress by neutralizing those free radicals (Al-Laith, 2010; Rashid et al., 2013; Sarr et al., 2015). Medicinal plants have antioxidant, anti-inflammatory, antimicrobial and therapeutic activity, thus playing an important role in health care and livelihood of people (Bouزيد et al., 2011; Prasathkumar et al., 2021).

Herbal medicines made from roots, leaves and stem bark through such methods as decoction and infusion are still being used due to their availability, affordable price and possible advantage in terms of safety (Ullah et al., 2020; Ahad et al., 2021; Rahayu et al., 2020). Those effects are mostly due to the action of phytochemical compounds with medicinal potential (George et al., 2021; Hossen et al., 2022; Zheng et al., 2022). Therefore, it would be interesting to study active components and biological activities of *Vitex doniana* and *Detariummicrocarpum* and get an insight on how to develop potent antioxidant and anti-inflammatory agents. The aim of

this study is to evaluate the phytochemistry and biological activities of stem bark and leaf extract of *Detarium microcarpum* and *Vitex doniana* plants.

II. MATERIALS AND METHODS

➤ Sample Collection and Preparation

The parts of the plant (stem and leaf) were collected in Lifare Village in Fufore Local Government Area of Adamawa State, Nigeria. The fresh plant parts were then brought in a black polyethylene bag to Chemistry Laboratory, Modibbo Adama University, Yola where the parts were identified by the taxonomist, Dr. Usman Jauro of Botany Department, Modibbo Adama University, Yola where specimens number was given HMAU 111245 (stem bark) and HMAU 111249 (leaf and deposited in the herbarium.

Drying of different plant parts was done under shade in the chemistry laboratory. Fresh samples were spread out and stirred. Well dried samples were grounded to fine powder with the help of mortar and Pestle. Fine powders of the samples were weighed using analytical balance.

The plants samples were subjected to exhaustive extraction using soxhlet extractor. One hundred grams (100g) of each sample in Methanol was used in the extraction process which lasted for 6 hours. The crude extract of each sample was filtered and concentrated in an oven at 40°C.

➤ Phytochemical Screening of the Extracts

• Test for Alkaloids

A solution of picric acid was added 2mls of the extract. An orange coloration indicates the presence of alkaloids

• Test for Flavonoids

Sodium hydroxide test was used to test for the presence of flavonoids. 5g of the sample was weighed and detained completely with acetone. The mixture was warmed on water bath to evaporate the acetone. The residue where extracted with water on a water bath. The mixture was filtered and the filtrate was used for the test. 5ml of 10% sodium hydroxide was added to an equal volume of the detained water extract. A yellow solution indicates the presence of Flavonoid.

• Test for Terpenoid

Exactly 0.2g of each of the extracts was mixed with 2ml Chloroform and 3ml of concentrated sulphuric acid was added carefully to form a layer. A reddish-brown coloration of the interface formed indicates the presence of terpenoids.

- *Test for Saponins*

Froth test was done on the samples. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5minutes. The mixture was filtered and 2.5ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was shaken vigorously for about 30 seconds and allowed to stand for half an hour. Honey comb froth indicates the presence of Saponins.

- *Test for Tannins*

Two 2g of the sample was boiled in 50ml distilled water for 30 minutes on a hot plate. The mixture was filtered and a portion of the filtrate was diluted with sterile water in a ratio of 1:4 and 3 drops of 10% ferric chloride solution added. A blue or green coloration indicates the presence of tannins.

- *Test for Steroids*

Two 2ml of acetic anhydride was added to 0.5g of the extracts followed by 2ml of Sulphuric acid. A violet color turns to blue which indicates the presence of steroids.

- *Test for Cardiac Glycosides*

Exactly 1.5 ml of solvent extract was mixed with 2 ml of glacial acetic and a drop of ferric chloride solution was added followed by the addition of 1 ml of conc. H₂SO₄. A brown ring in the interface indicates the presence of doxy sugars of cardenoloides. A violet ring may appear beneath the brown ring while acetic layers and green ring may also form just gradually towards the layer.

- *Test for Anthraquinones*

To 5ml of extract, few ml of conc. H₂SO₄ was added and 1ml of diluted ammonia was added to it. The appearance of rose pink confirms the presences of anthraquinones

➤ *Antioxidant Assay;*

1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay was employed for Antioxidant assay.

DPPH radical scavenging activity was determined as described by Molyneux (2004). 1 ml of 0.2 mM DPPH was prepared with methanol and added to 2.5 ml solution of varying concentrations of the various methanolic crude extract and ascorbic acid as standard at different concentrations of 20, 40, 60, 80 and 100 µg/mL and allowed to react at room temperature in darkness for 30 min. 1 ml of 0.2 mM DPPH and 2.5 ml methanol was serve as a control. This assay was carried out in triplicates for each concentration. Absorbance of the resultant mixture was measured using the double beam UV-visible spectrophotometer (Model T80; PG Instruments, Lutterworth, England, UK) at 517 nm. Percentage inhibitions of the methanol extract and the standard will be calculated using the formula below:

$$\% \text{ inhibition} = \frac{\text{Optical Density Control} - \text{Optical Density sample}}{\text{Optical Density control}} \times 100$$

Optical Density control

Optical Density control = The absorbance without sample,
Optical Density sample = The absorbance of methanol extract or standard.

Percentage inhibition indicates the capacity of fractions to inhibit reactive oxygen species, and the concentration of sample required for 50% inhibition was determined and expressed as an IC₅₀ value.

➤ *Antimicrobial screening*

The antimicrobial screening of *Vitex dioniana* and *Detarium microcarpum* extracts was conducted using the agar was diffusion method, which is widely used for preliminary in vitro assessment of antimicrobial activity. The plant extracts—prepared via solvent extraction using methanol, ethanol, or aqueous solutions—was tested against a panel of clinically relevant microorganisms, including both Gram-positive and Gram-negative bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as fungal strains like *Candida albicans*. Sterile Mueller-Hinton agar (for bacteria) and Sabouraud dextrose agar (for fungi) was inoculated with the microbial suspensions, and wells was bored into the agar medium to introduce the plant extracts. Standard antibiotics such as ciprofloxacin (for bacteria) and fluconazole (for fungi) was serve as positive controls, while solvents used in extraction was act as negative controls. After incubation at 37°C for 24 hours (for bacteria) and 28°C for 48 hours (for fungi), zones of inhibition will be measured to determine antimicrobial potency. The results was statistically analyzed, and Minimum Inhibitory Concentration (MIC) values may be further determined using broth dilution methods for the most active extracts, following protocols outlined by the Clinical and Laboratory Standards Institute (CLSI, 2020).

➤ *In vitro anti-inflammatory activity*

Inhibition of albumin denaturation. The 5ml of reaction mixture was comprised of 0.2ml of eggs albumin, 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentration of extracts. Similar volume of double distilled water was served as control. Then the mixture was incubated at 37 °C in incubator for about 15mins and then heated at 70 °C for 5mins. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac sodium (standard drug) was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula mentioned below.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100$$

III. RESULTS AND DISCUSSION

➤ Phytochemical Screening of Extract of the Plants

The phytochemical screening of *Detarium microcarpum* and *Vitex doniana* (leaves and stem) revealed the presence of

alkaloids, saponins, phenolics/tannins, steroids, and glycosides, while flavonoids, terpenoids, anthraquinones are shown in Table 1.

Table 1: Phytochemical Screening of Extract of the Plants

Phytochemical	DM Leaves	DM Stem	VD Leaves	VD Stems
Alkaloids	+	+	+	+
Flavonoids	+	+	-	-
Terpenoids	+	+	-	+
Saponins	+	-	+	+
Tannins	-	+	+	-
Steroids	-	+	+	-
Glycoside	+	+	+	+
Anthraquinone	+	-	-	-

Keys: DM = *Detarium microcarpum* VD = *Vitex doniana* + = Present - = Absent

Phytochemical screening revealed that *Vitex doniana* leaves contained alkaloids, saponins, tannins, steroids, and glycosides, while the stem showed a wider range of compounds including terpenoids, suggesting greater secondary metabolite accumulation. *Detarium microcarpum* leaves contained alkaloids, flavonoids, terpenoids, saponins, glycosides and anthraquinones, indicating potential antioxidant, antimicrobial, and anti-inflammatory activities. The stem

extract contained alkaloids, flavonoids, tannins, steroids, and glycosides, with tannins and flavonoids contributing to its reported antimicrobial effects. Overall, both plants exhibited rich phytochemical profiles that support their traditional medicinal uses and potential for further pharmacological investigations.

➤ Antioxidant Activity of the Extracts

Table 2: Antioxidant Activity of *Vitex doniana* Stem and Leaves Extract

Sample Name	Stem		Leaves	
	517.0nm	Percentage Inhibition (%)	517.0nm	Percentage Inhibition (%)
Blank	0.3729		0.1509	59.5
<i>Vitex doniana</i> 20	0.0756	79.9	0.0897	75.9
<i>Vitex doniana</i> 40	0.0526	85.9	0.0737	80.2
<i>Vitex doniana</i> 60	0.0253	93.2	0.0324	91.3
<i>Vitex doniana</i> 80	0.0538	85.5	0.0352	90.5
<i>Vitex doniana</i> 100	0.0372	90.0	0.0365	90.2
<i>Vitex doniana</i> 200	0.0567	84.8		

The data in Table 2 demonstrate that the stem extract of *Vitex doniana* exhibited significant antioxidant activity across all concentrations tested. percentage inhibition increased from 79.9% at 20 µg/mL to a peak of 93.2% at 60 µg/mL, indicating a strong concentration-dependent effect. The fluctuations at higher concentrations, such as the slight reduction at 80 µg/mL (85.5%) and 200 µg/mL (84.8%), may reflect experimental

variability or saturation of free radical scavenging sites. Table 2 shows that the leaf extract of *Vitex doniana* had robust antioxidant effects, with inhibition increasing from 59.5% at 20 µg/mL to a maximum of 91.3% at 80 µg/mL. Percentage inhibition remained high across subsequent concentrations, indicating strong free radical scavenging ability even at elevated doses.

Table 3: Antioxidant Activity of *Detarium microcarpum* Stem and Leaves Extract

Sample Name	Stem		Leaves	
	517.0nm	Percentage Inhibition (%)	517.0nm	Percentage Inhibition (%)
<i>Vitex doniana</i> 20	0.3138	15.8	0.1438	61.4
<i>Vitex doniana</i> 40	0.2707	27.4	0.0218	94.2
<i>Vitex doniana</i> 60	0.1996	46.5	0.0164	95.6
<i>Vitex doniana</i> 80	0.1728	53.7	0.0252	93.2
<i>Vitex doniana</i> 100	0.1162	68.8	0.0296	92.1
<i>Vitex doniana</i> 200	0.0540	85.5	0.0343	90.8

According to Table 3, the stem extract of *Detarium microcarpum* displayed a gradual, concentration-dependent increase in antioxidant activity, from 15.8% at 20 µg/mL to 85.5% at 200 µg/mL. The dose-dependent trend underscores the importance of concentration optimization in antioxidant applications and supports further phytochemical analysis to

identify and quantify the specific compounds responsible for radical inhibition. Table 3 indicates that the leaf extract of *Detariummicrocarpum* has exceptionally strong antioxidant activity, with percentage inhibition ranging from 61.4% at 20 µg/mL to 95.6% at 60 µg/mL.

Table 4: Antimicrobial Sensitivity of *Detarium microcarpum* Leaf Extract Against Selected Microorganisms (mm)

S/n	Organisms	Concentrations				Positive Control	
		100ug/ml	75ug/ml	50ug/ml	25ug/ml	Aug	Keto
	<i>S. aureus</i>	16	13	9	7	30mg 20	-
	<i>S.epidermidis</i>	11	9	6	6	30ug 19	-
	<i>E. Coli</i>	9	8	7	6	30ug 23	-
	<i>S. typhi</i>	18	13	12	10	30ug 26	-
	<i>C. albicans</i>	14	11	9	8	-	50ug 25
	<i>A. niger</i>	17	12	8	6	-	50ug 21

Antimicrobial activity of the plant extract increased with concentration from 25 µg/mL to 100 µg/mL, showing a clear dose-dependent effect linked to higher levels of active phytochemicals. Among the tested bacteria, *Salmonella* species showed the greatest susceptibility, followed by *Staphylococcus aureus*, while *Escherichia coli* was least affected, likely due to its outer membrane barrier that limits uptake of plant-derived compounds. The fungal isolates exhibited moderate sensitivity, with *Aspergillus niger* showing noticeable inhibition at higher concentrations, indicating antifungal potential. These findings align with previous reports on *Detarium microcarpum* leaves, where antimicrobial activity has been attributed to bioactive

constituents such as flavonoids, tannins, and phenolic compounds. Although the extract was less potent than standard antibiotics and antifungals, its broad-spectrum inhibitory effects support its traditional use in managing infectious diseases.

➤ Antimicrobial Sensitivity of *Detarium microcarpum* Stem Extract against Selected Microorganisms

Results presented in Table 5 indicate that the stem extract of *Detariummicrocarpum* also exhibited antimicrobial properties, though its effectiveness was generally lower than that of the leaf extract.

Table 5: Antimicrobial Sensitivity of *Detarium microcarpum* Stem Extract against Selected Microorganisms (mm)

S/n	Organisms	Concentrations				Positive Control	
		10ug/ml	75ug/ml	50ug/ml	25ug/ml	Aug	Keto
	<i>S. aureus</i>	11	6	6	6	30ug 23	-
	<i>S.epidermidis</i>	12	9	8	7	30ug 21	-
	<i>E. Coli</i>	7	6	6	6	30 ug 23	-
	<i>S. typhi</i>	13	11	9	7	30ug 12	-
	<i>C. albicans</i>	11	9	6	6	-	50ug 19
	<i>A. niger</i>	14	11	9	8	-	50ug 17

An incremental increase in inhibition zones was observed with rising concentrations, confirming a concentration-dependent response pattern. The stem extract was most effective against *Salmonella* species and *Staphylococcus epidermidis*, while *E. coli* displayed minimal inhibition. This reduced activity against *E. coli* aligns with previous findings attributing resistance in Gram-negative bacteria to their complex cell envelope structure (Silhavy et al., 2010). Fungal organisms, particularly *Aspergillus niger*, showed moderate

sensitivity, though the degree of inhibition was comparatively smaller than that recorded for leaf extracts.

➤ Antimicrobial Sensitivity of *Vitex doniana* Leaf Extract against Selected Microorganisms

As shown in Table 6, the leaf extract of *Vitex doniana* exhibited pronounced antimicrobial activity across a wide range of tested organisms.

Table 6: Antimicrobial Sensitivity of *Vitex doniana* Leaf Extract Against Selected Microorganisms (mm)

S/n	Organisms	Concentrations				Positive Control	
		100ug/ml	75ug/ml	50ug/ml	25ug/ml	Aug	Keto
	<i>S. aureus</i>	19	16	13	9	30ug 26	-
	<i>S.epidermidis</i>	14	11	9	7	30ug 20	-
	<i>E. Coli</i>	13	9	8	7	30ug 21	-
	<i>S. typhi</i>	12	9	8	6	30ug 16	-
	<i>C. albicans</i>	16	13	9	8	-	50 ug 21
	<i>A. niger</i>	8	6	6	6	-	50ug 18

Substantial inhibition zones were observed even at lower concentrations, indicating strong intrinsic antimicrobial potency. *Staphylococcus aureus* emerged as the most susceptible bacterial species, followed closely by *Staphylococcus epidermidis*, *Salmonella* species, and *Escherichia coli*. The broad antibacterial activity observed may be attributed to the presence of multiple bioactive compounds capable of disrupting microbial cell membranes, enzymes, or genetic material. At higher concentrations, the inhibition produced by the extract approached that of ketoconazole.

Although *Aspergillus niger* was less sensitive, the observed inhibition confirms antifungal potential. These findings provide experimental validation for the widespread traditional use of *V. doniana* leaves in managing infectious conditions.

➤ *Antimicrobial Sensitivity of Vitex doniana Stem Extract against Selected Microorganisms*

The antimicrobial performance of *Vitex doniana* stem extract, as summarized in Table 7, revealed consistent inhibitory activity across all test organisms.

Table 7: Antimicrobial Sensitivity of *Vitex doniana* Stem Extract Against Selected Microorganisms (mm)

S/n	Organisms	Concentrations				Positive Control	
		500ug/ml	250ug/ml	125ug/ml	6.25ug/ml	Aug	Keto
	<i>S. aureus</i>	12	9	8	7	30ug 22	-
	<i>S.epidermidis</i>	13	11	6	6	30ug 30	-
	<i>E. Coli</i>	14	13	10	8	30ug 20	-
	<i>S. typhi</i>	11	8	7	6	30ug 21	-
	<i>C. albicans</i>	15	13	11	9	-	50ug 26
	<i>A. niger</i>	16	12	9	6	-	50ug 13

Inhibition zones expanded progressively as the extract concentration increased from 6.25 µg/mL to 500 µg/mL, demonstrating a strong dose response relationship. Among bacterial isolates, *Staphylococcus epidermidis* and *Escherichia coli* were particularly responsive, suggesting that the stem extract contains compounds effective against both Gram-positive and Gram-negative bacteria. The fungal species evaluated, including *Candida* species and *Aspergillus niger*, also showed measurable growth suppression, indicating that the stem extract possesses antifungal constituents. Although the antimicrobial activity of the stem extract remained lower than

that of conventional antimicrobial agents, its broad-spectrum action highlights its medicinal relevance.

➤ *Anti-Inflammatory Activity*

• *In Vitro Anti-Inflammatory Activity of Vitex doniana Stem Extract (V.D.S)*

Analysis of the data in Table 8 shows that the stem extract of *Vitex doniana* produced a progressive enhancement of anti-inflammatory activity with increasing concentration.

Table 8: In Vitro Anti-Inflammatory Activity of *Vitex doniana* Stem Extract (V.D.S)

Sample	Concentration (µg/mL)	Absorbance 1	Absorbance 2	Mean Absorbance	SEM (±)	% Inhibition (Mean)
Negative Control	–	0.258	0.258	0.258	0.000	0.00
Extract	10	0.220	0.222	0.221	0.001	14.34
Extract	50	0.175	0.177	0.176	0.001	31.78
Extract	100	0.125	0.123	0.124	0.001	51.94
Extract	500	0.055	0.055	0.055	0.000	78.65
Diclofenac Sodium	10	0.165	0.167	0.166	0.001	35.66
Diclofenac Sodium	50	0.089	0.091	0.090	0.001	65.12
Diclofenac Sodium	100	0.026	0.028	0.027	0.001	89.53

A steady decline in absorbance values was recorded as the extract dose increased from 10 to 500 µg/mL, which translated into a corresponding rise in inhibition from 14.34% to 78.65%. This concentration-related response suggests that the inhibitory effect of the extract is directly influenced by dosage. The minimal SEM values obtained across the tested concentrations further indicate a high degree of experimental consistency. Although diclofenac sodium exhibited greater inhibitory potency, the stem extract demonstrated considerable activity at higher concentrations, implying the presence of biologically active compounds. Previous investigations have reported that *Vitex doniana* stems contain phenolic compounds and flavonoids capable of modulating inflammatory pathways

through suppression of mediators such as cyclooxygenase and nitric oxide (Burkill, 1997; Adebayo et al., 2019). Comparable dose-responsive anti-inflammatory effects have also been observed in related *Vitex* species, reinforcing the pharmacological relevance of the present findings (Onoja et al., 2017).

• *In Vitro Anti-Inflammatory Activity of Vitex doniana Leaf Extract (V.D.L)*

The results presented in Table 9 indicate that the leaf extract of *Vitex doniana* exerted a pronounced inhibitory effect on inflammatory activity in vitro.

Table 9: In Vitro Anti-Inflammatory Activity of *Vitex doniana* Leaf Extract (V.D.L)

Sample	Concentration (µg/mL)	Absorbance 1	Absorbance 2	Mean Absorbance	SEM (±)	% Inhibition (Mean)
Negative Control	–	0.258	0.258	0.258	0.000	0.00
Extract	10	0.245	0.243	0.244	0.001	5.43
Extract	50	0.210	0.212	0.211	0.001	18.22
Extract	100	0.140	0.138	0.139	0.001	46.12
Extract	500	0.046	0.046	0.046	0.000	82.13
Diclofenac Sodium	10	0.165	0.167	0.166	0.001	35.66
Diclofenac Sodium	50	0.089	0.091	0.090	0.001	65.12
Diclofenac Sodium	100	0.026	0.028	0.027	0.001	89.53

Percentage inhibition increased markedly from 5.43% at the lowest concentration to 82.13% at 500 µg/mL, reflecting improved suppression of inflammatory reactions with increasing dose. The gradual reduction in absorbance values and the consistency of SEM readings further validate the reliability of the assay. Compared with the stem extract, the leaf extract showed superior inhibitory performance at equivalent concentrations, particularly at higher doses. This enhanced activity may be attributed to the higher accumulation of phytochemicals in the leaves. Earlier studies have identified *Vitex doniana* leaves as a rich source of flavonoids, alkaloids, and polyphenols, compounds widely recognized for their anti-

inflammatory and antioxidant functions (Egharevba et al., 2015; Adewale et al., 2021). The strong activity observed in this study therefore supports existing ethnomedicinal claims and highlights the leaves as a promising candidate for anti-inflammatory drug development.

• *In Vitro Anti-Inflammatory Activity of Detarium microcarpum Stem Extract (D.M.S)*

Data in Table 10 revealed that the stem extract of *Detarium microcarpum* demonstrated a measurable yet moderate anti-inflammatory response.

Table 10 In Vitro Anti-Inflammatory Activity of *Detarium microcarpum* Stem Extract (D.M.S)

Sample	Concentration (µg/mL)	Absorbance 1	Absorbance 2	Mean Absorbance	SEM (±)	% Inhibition (Mean)
Negative Control	–	0.258	0.258	0.258	0.000	0.00
Extract	10	0.240	0.242	0.241	0.001	6.59
Extract	50	0.215	0.217	0.216	0.001	16.28
Extract	100	0.185	0.187	0.186	0.001	27.91
Extract	500	0.119	0.119	0.119	0.000	53.72
Diclofenac Sodium	10	0.165	0.167	0.166	0.001	35.66
Diclofenac Sodium	50	0.089	0.091	0.090	0.001	65.12
Diclofenac Sodium	100	0.026	0.028	0.027	0.001	89.53

Inhibition values increased from 6.59% at 10 µg/mL to 53.72% at 500 µg/mL, indicating a gradual concentration-dependent effect. Despite this trend, the overall activity remained lower than that recorded for *Vitex doniana* extracts. When evaluated alongside diclofenac sodium, the stem

extract exhibited comparatively weak inhibition, suggesting limited potency. Previous reports have similarly indicated that the stem portions of *Detarium microcarpum* generally display lower pharmacological activity than other plant parts, likely due to reduced levels of bioactive constituents (Lockett et al., 2000;

Ajayi et al., 2018). Nonetheless, the observed inhibitory effect implies the presence of anti-inflammatory compounds that merit further isolation and characterization.

➤ *In Vitro Anti-Inflammatory Activity of Detarium microcarpum Leaf Extract (D.M.L)*

As shown in Table 11, the leaf extract of *Detariummicrocarpum* produced a clear dose-related anti-inflammatory effect.

Table 11: In Vitro Anti-Inflammatory Activity of *Detarium microcarpum* Leaf Extract (D.M.L)

Sample	Concentration (µg/mL)	Absorbance 1	Absorbance 2	Mean Absorbance	SEM (±)	% Inhibition (Mean)
Negative Control	–	0.258	0.258	0.258	0.000	0.00
Extract	10	0.180	0.182	0.181	0.001	29.84
Extract	50	0.145	0.143	0.144	0.001	44.19
Extract	100	0.115	0.117	0.116	0.001	55.04
Extract	500	0.100	0.100	0.100	0.000	61.24
Diclofenac Sodium	10	0.165	0.167	0.166	0.001	35.66
Diclofenac Sodium	50	0.089	0.091	0.090	0.001	65.12
Diclofenac Sodium	100	0.026	0.028	0.027	0.001	89.53

Inhibition increased from 29.84% at the lowest concentration to 61.24% at 500 µg/mL, accompanied by a consistent decrease in absorbance readings. This response pattern indicates that the leaves possess greater anti-inflammatory potential than the stem. Although the activity of diclofenac sodium remained superior, the leaf extract demonstrated appreciable efficacy, particularly at lower concentrations. This observation aligns with earlier findings that attribute the medicinal value of *D. microcarpum* leaves to their high content of flavonoids and phenolic compounds, which are known to attenuate inflammatory processes (Oibiokpa et al., 2014; Auwal et al., 2020). Collectively, these results provide scientific backing for the traditional use of the plant leaves and justify further pharmacological investigations.

IV. CONCLUSION

The study established that *Vitex doniana* and *Detarium microcarpum* possess valuable biological properties due to their phytochemical constituents. The antioxidant, antimicrobial, and anti-inflammatory activities observed indicate that these plants may serve as promising sources of natural therapeutic compounds.

➤ *Conflict of Interest*

Authors has declared that no competing interest exist.

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