

Chemical Profiling, Antioxidant and Antimicrobial Activity of *Cinnamomum Tamala* (Indian Bay Leaf) Extract

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Abstract: Nearly 80% of the world's population still relies on traditional medicines for primary health care, most of which involve the use of plant extracts which mostly contain bioactive compounds. The chemical composition and antioxidant activity of the ethanol leaf extract of *Cinnamomum tamala* were investigated using Gas Chromatography-Mass Spectrometry (GC-MS) and 2,2-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging and ferric reducing ability of plasma model (FRAP) respectively. Crude extract of *C. tamala* (bay leaf) was also investigated for its antimicrobial properties using the well in agar diffusion method. The results obtained from GC-MS analysis indicated that 35 compounds are present in the leaf extract. The most abundant compounds identified for their % peak areas were benzoic acid (55.8%), benzyl benzoate (27.7%), 2,2,3,3,6,6,7,7 octahydrobenzaldehyde (2.02%), *n*-hexadecanoic acid (1.62%), 2,6-dimethoxy-4-(-2-propenyl) phenol (1.13%), diethyl phthalate (1.11%) (-) – spathulenol (1.02%). The result of the antioxidant activity of the crude extract of bay leaf revealed a dose-dependent scavenging of DPPH as well as the ability of the extract to reduce $FeCl_3$ solution. On comparing the results obtained in this study with vitamin C which was used as positive control, it was observed that *C. tamala* possessed significant antioxidant ability which may be attributed to the presence of oxygenated and polyphenolic compounds in the extract. The susceptibility of these isolates towards the leaf extract was compared with gentamycin and nystatin, which were used as a positive control for bacteria and fungi respectively. Results obtained showed that the extract was

able to inhibit the growth of the isolates at various concentrations. However, no antimicrobial activity was recorded for *Salmonella* sp. and *Escherichia coli* at different concentrations, indicating that they were resistant to *C. tamala* extract. On comparing the zones of inhibition of the extracts with that of the standard (Gentamycin and nystatin) for bacteria and fungi respectively, the results showed that the zone of inhibition of the standard for all the tested isolates was greater than that of the extracts.

Keywords: *Cinnamomum tamala*, 2, 2-diphenyl-2-picryl hydrazyl, benzoic acid, gas chromatography-mass spectrometry

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1.0 Introduction

Plants have been a primary source of medicinal agents for centuries, and many modern drugs have been developed from bioactive compounds found in natural sources (Newman

& Cragg, 2020). Spices, in particular, have played a dual role as both food flavouring agents and sources of therapeutic compounds. One such spice is *Cinnamomum tamala* (Indian bay leaf), an aromatic, evergreen plant belonging to the *Lauraceae* family. Indigenous to the Indian subcontinent and parts of Southeast Asia, *C. tamala* has long been used in traditional medicine for its antimicrobial, anti-inflammatory, and antioxidant properties (Bhargavi et al., 2024; Sharma et al., 2009).

Cinnamomum tamala is rich in bioactive compounds, including flavonoids, polyphenols, essential oils, and terpenoids, which contribute to its pharmacological potential (Bisht, 2021; Leela, 2008). The leaves contain volatile components such as eugenol, linalool, and cinnamaldehyde, which have demonstrated antimicrobial and antioxidant activities in previous studies (Kumar et al., 2012; Roberts & Moreau, 2016). These properties make *C. tamala* a promising candidate for applications in food preservation, pharmaceuticals, and natural health products.

Several studies have examined the phytochemical composition and medicinal benefits of *Cinnamomum* species. Research on *Cinnamomum verum* has highlighted its strong antioxidant and antibacterial activities due to its high phenolic and flavonoid content (Ayodeji et al., 2022). Similarly, *C. tamala* has demonstrated antimicrobial properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Kaefer & Milner, 2011; Virendra et al., 2012). However, limited research has been conducted on its effectiveness against a broader range of bacterial and fungal pathogens. While the antioxidant potential of *C. tamala* has been explored using methods such as DPPH radical scavenging, comparative studies with standard antioxidants like vitamin C remain scarce (Re et al., 2000).

Despite the abundance of bioactive compounds in *C. tamala*, its application in Nigeria remains underexplored. The plant is primarily

imported, and its local cultivation is minimal, with only a small percentage of the population—mostly educational institutions—engaging in its research and cultivation (Ayodeji et al., 2022). This presents an opportunity to assess its potential for domestic cultivation and industrial applications.

This study investigates the chemical composition, antioxidant, and antimicrobial activities of *Cinnamomum tamala* leaf extract. The research focuses on determining the phytochemical composition of the extract using Gas Chromatography-Mass Spectrometry (GC-MS), evaluating its antioxidant activity through DPPH radical scavenging and ferric reducing ability of plasma (FRAP) assays, and assessing its antimicrobial activity against selected bacterial and fungal strains using the agar diffusion method. The study also compares the antimicrobial efficacy of the extract with standard antibiotics such as gentamycin and nystatin and evaluates its antioxidant capacity relative to vitamin C.

The findings of this study will contribute to the growing body of knowledge on the medicinal potential of *Cinnamomum tamala*. By elucidating its phytochemical profile and bioactivities, this research could facilitate its application in developing natural antimicrobial agents, antioxidant supplements, and food preservatives. Additionally, understanding its potential against multidrug-resistant pathogens may provide new avenues for addressing antibiotic resistance (Newman & Cragg, 2020). The study also aims to highlight the need for local cultivation and commercial utilization of *C. tamala* in Nigeria, reducing reliance on imported bay leaves and promoting the development of indigenous herbal medicine industries.

Given the global shift towards natural and plant-derived therapeutics, *Cinnamomum tamala* holds significant promise as a source of bioactive compounds with pharmacological potential. However, further studies are required



to explore its full medicinal applications, optimize extraction methods, and assess its efficacy in clinical settings. This study provides a foundation for future research into its potential uses in food, medicine, and industrial applications.

2.0 Materials and Methods

2.1 Sample Collection/Identification

The leaves of *C. tamala* used for the study were collected at Afaha Etuk Ibesikpo in Akwa Ibom State of Nigeria in May 2023. The plant was identified and authenticated. The voucher herbarium specimens were deposited at the herbarium at the faculty of pharmacy, University of Uyo. (Voucher no. UUPH 3a (b).

2.2 Sample Preparation

The leaves were washed, dried, ground and stored in a well-labelled air-tight container. The whole powdered leaf was macerated with ethanol in a stoppered container for 48 hours with frequent agitation. This was followed by filtration and evaporation in a rotary vacuum evaporator to obtain crude extract.

2.3 Percentage yield

The percentage yield of *C. tamala* was calculated and given as equation 1

$$\% \text{ yield} = \left(\frac{\text{weight of extract}}{\text{weight of the dried sample}} \right) \times \frac{100}{1} \quad (1)$$

2.4 Gas Chromatography-Mass spectrometry

The extracts were subjected to GC-MS analysis on an Agilent system consisting of a model 7890N gas chromatography, and a model mass detector Triple Quad 7000 A in Electron Impact I) mode at 70 eV (m/z range 400-600 amu). The GC column was HP-5ms fused silica capillary with a 5%phenyl-methyl polysiloxane stationary phase 130 m × 250µm × 0.25µm. The carrier gas was helium with a column head pressure of 9.783 psi and flow flavours 1.2ml/min inlet temperature and MSD detector temperature 250°C.

The components were identified by comparison of their mass spectra with NIST

1998 library data of the GC-MS system. The relative amount of each component of the extract was obtained as the percentage of the peak area. The retention indices value of each component was determined relative to the retention time of homologous n-alkane series with linear interpolation on the HP- 5ms column.

2.5 Antioxidant Assay

2,2-diphenyl-2-picryl hydrazyl (DPPH) Radical scavenging Assay

DPPH free radical scavenging of *Cinnamomum tamala* leaf extract and L-ascorbic acid prepared in methanol at concentrations (150-950µg/l) were evaluated according to the method of Choi *et al.* (2002). 200µl of 0.3mM of DPPH solution in methanol was mixed with 150µl of solutions prepared with extracts and standard. The mixtures were well-shaken and kept at room temperature in the dark for 30 minutes. The absorbance was measured at 518 nm using a UV visible spectrophotometer. The radical scavenging activity (RSA) is calculated as a percentage of DPPH radical discolouration using equation 2

$$\%RSA = \left(\frac{A_0 - A_s}{A_0} \right) \times \frac{100}{1} \quad (2)$$

where A_0 = Absorbance of Blank, A_s = Absorbance of Sample

Ferric Reducing/Antioxidant Power (FRAP) Assay

The reducing property was determined by accessing the ability of the extract of *C. tamala* to reduce FeCl_3 solution as described by Benzie and Strain, (1996) with modifications.

FRAP solution (3.5 ml) was added to 0.4 ml of distilled water and incubated at 37 °C for 5 minutes. Then this solution was mixed with different concentrations of the plant extract (0.1, 0.4, 0.8, 1.0, 1.12, 1.5 ml) and incubated at 37°C for 8 minutes. The absorbance of the reaction mixture was measured at 593nm. For the construction of the calibration curve, 5 concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1, 0.4, 0.8,



1.0, 1.12, 1.5.ml) were used and the absorbance values were measured as for sample solutions.

2.6 Antimicrobial assay of plant extract

2.6.1 Collection of microbial test organisms

The organisms included three (3) gram-positive (G+ve), five (5) gram-negative (G-ve), one (1) yeast and one (1) mould. (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella sp*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella sp*, *Shigella sp*, *Candida albicans*, and *Aspergillus niger*). These organisms were obtained from the Microbiology stock culture unit, the University of Uyo. Isolates were sub-cultured and preserved as pure cultures on Nutrient agar and Sabourad Dextrose agar slants and stored at low temperatures until required.

2.6.2 Preparation of test organisms used for the work.

G+ve and fungi test organisms were serially diluted using 10-fold dilution to factor three and G-ve to factor five and thereafter the last dilutions compared with the McFarland standard.

2.6.3 Determination of extract concentrations

The extract was dissolved using sterile water to constitute a different concentration of 20, 40, 60.80 and 100mg/ml

2.6.4 Determination of antimicrobial assay of plant extracts

The antimicrobial activity of the extracts was evaluated using the well-in agar diffusion technique as described by Okeke *et al.*, (2001). Test organisms were diluted using Nutrient broth and Malt Extract broth for bacterial and isolates respectively. They were further sub-cultured into Peptone water and cells adjusted to the McFarland Turbidity standard. 0.1ml of each diluted test organism was aseptically transferred and spread on the surface of the Muller Hinton Agar (MHA), sterile swab sticks were used to spread the inoculum on the

surface of the medium and allowed them to dry on the bench.

A sterile cork borer of 5 mm was used to bore holes on the surface of the medium that were seeded with the test organisms. In each of the wells previously seeded with the test organisms, 0.2 ml of the extract dilution concentrations were introduced into the wells. Control experiments were set up alongside the extracts using commercial antibiotics and antifungal drugs (Gentamycin and Nystatin) 10mg/ml and 40 mg/ml for bacterial and fungal respectively.

All plates were left on the bench for 1 hour before incubating at 37 °C for 24 hours for bacterial and 28 °C for 5 days for fungal isolates respectively. After incubation, antimicrobial activities were determined by measuring the Incubation Zone Diameter (IZD) in all the activities of the extracts.

3.0 Results and Discussion

3.1 Percentage yield

Extraction yield quantifies the efficiency of a solvent in isolating specific components from a given material (Osman *et al.*, 2019). It is influenced by factors such as solvent polarity, pH, temperature, extraction time, and sample composition. In this study, the ethanol extract of *Cinnamomum tamala* leaves yielded 26.88%, a moderately high value, likely due to the presence of predominantly polar compounds.

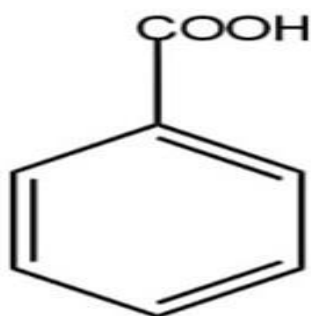
3.2 GC-MS spectroscopy of leaf extract of *C. tamala*

The ethanol leaf extract of *C. tamala* was analyzed to ascertain the bioactive compounds present using GC-MS. Thirty-five (35) compounds were identified in the extract comprising alkanes, alkenes, alcohols, terpenoids, saturated and unsaturated fatty acids, fatty acid esters and aromatic hydrocarbons. The identified compounds, their retention indexes and the percentage composition of each compound are given in



Table 1 and Fig. 1. The most abundant compounds identified for their % peak areas were benzoic acid (55.8%), benzyl benzoate (27.9%), spathulenol (1.1%), Di ethyl phthalate (1.11%), 2, 6 dimethoxy-4 (2-propyl) phenol, (1.13%), and n-hexadecanoic acid (1.62%).

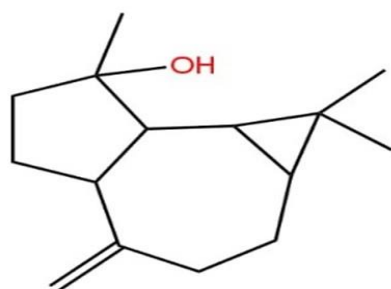
Benzoic acid was the major constituent present in the leaf extract of *C. tamala* (55.8%). It is an organic compound used commercially in the production of dyes, plastics etc (Gaur, 2008; Aggarwal *et al.*, 2020).



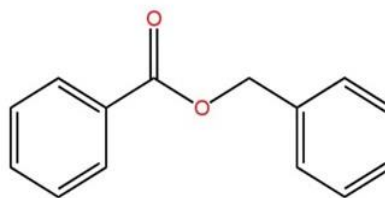
Benzoic acid

The presence of benzoic acid in bay leaf corroborates its use in dye production as reported by Tiwari and Talreja, (2020).

(-)-Spathulenol is a tricyclic sesquiterpene alcohol which has a basic skeleton similar to azulenes. It occurs in oregano among other plants including *C. tamala*. Due to its high and strong scents, it is used in the manufacture of perfumes (Mir *et al.*, 2004).

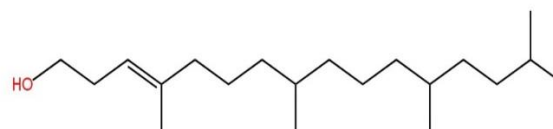


(-)-Spathulenol



Benzyl benzoate

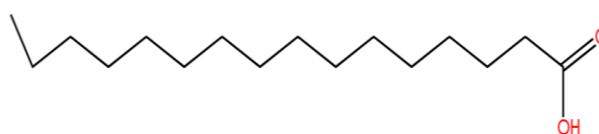
Benzyl benzoate is also an organic compound present in the extract (27.9%). It is used as an insect repellent, and dyes. It is also used in skincare products and has antibacterial effects against Scabies and lice (Tiwari and Talreja, 2020).



Phytol

Phytol is an acyclic diterpene alcohol and a constituent of chlorophyll. It is commonly used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1, it is an aromatic ingredient used in many fragrance compounds and it may be found in cosmetic and non-cosmetic products. It has antioxidant activities (Rani *et al.*, 2017).

N-Hexadecanoic acid commonly known as Palmitic acid has anti-alopecic, nematocidal, pesticidal, antioxidant and anti-androgenic properties. They also act as hemolytic 5-alpha reductase inhibitors and lubricants, and also possess hypocholesterolemic properties, (Komansilan *et al.*, 2012, Isaiah *et al.*, 2016).



Hexadecanoic acid

Table 1: Result of GC-MS analysis of *Cinnamomum tamala* leaf extract



PK	RT	% Composition	Constituents	Ref	CAS	Qual
1	2.924	0.54	Ethyl ester	25144	000093-89-0	95
2	2.993	1.26	Ethyl ether	25140	000093-89-0	94
3	3.833	55.8	Benzoic acid	10071	000065-87-0	95
4	4.039	0.36	3-methoxy acetophenone	25120	000586-37-8	72
5	4.303	0.21	α – <i>copaene</i>	68474	003856-25-5	98
6	4.628	0.37	Caryophyllene	68509	000087-44-5	95
7	4.753	0.13	Oxepine	10132	001487-99-6	70
8	4.879	0.11	Isoeugenol	34688	005932-68-2	38
9	5.068	0.90	Azulene	68912	022567-17-5	95
10	5.211	0.15	Naphthalene	68798	000483-76-1	98
11	5.59	0.31	9 H-fluorene	48063	002525-37-7	50
12	5.656	1.11	Di ethyl phthalate	84099	000084-66-2	98
13	5.982	1.02	(-) – Spathulenol	83504	1077171-55-2	91
14	6.319	0.10	Methyl tetradecanoate	104286	000124-10-7	97
15	6.382	1.13	2,6- dimethoxy-4- (2-propenyl) phenol	59289	006627-88-9	78
16	6.571	0.16	α – <i>nitroacetophenone</i>	35893	000614-21-1	38
17	6.691	0.22	Tetradecanoic acid	91417	000544-63-8	97
18	6.874	27.97	Benzyl benzoate	76381	0001120-51-4	87
19	7.051	0.09	3, 7, 11, 15-tetramethyl-2 hexa decen-1- ol	155865	102608-53-7	43
20	7.148	0.16	1,12-tridecadiene	48006	1021964-48-7	55
21	7.240	0.49	2-phenyl ethyl ester	89682	000094-47-3	90
22	7.337	0.10	2- hydroxy-phenyl methyl ester	91333	000118-58-1	53
23	7.394	0.621	Hexadecanoic acid	130822	000112-39-0	99
24	7.702	1.62	n-hexadecanoic acid	117419	000057-10-3	99
25	8.257	0.36	Octa- decenoic	155721	002462-84-2	99
26	8.320	0.45	Phytol	155849	000150-86-7	98
27	8.571	0.54	9,12,15, Octadecatrienoic acid	138418	000463-40-1	99
28	8.628	0.20	11-octadecanoic acid	144272	000057-11-4	99
29	10.16	0.14	Bis (2 ethyl hexyl) phthalate	233372	000117-81-7	96
30	10.40	0.32	Phenyl methyl ester	2022891	047557-83-5	76
31	11.40	0.22	9,12- Octadecadienoic acid	221096	041755-60-6	99
32	11.46	0.13	8 H –naphthol	165661	055836-76-5	25



33	12.97 2	2.02	2, 2', 3, 3', 6, 6', 7, 7' octahydrobenzylaldehyde	201262	000095-01-2	50
34	13.77 8	0.08	Eugenol	126588	1000394-92-1	58
35	14.87 5	0.17	Tocopherol	250953	010191-41-0	95

where; PK = Number of peaks, RT = Retention time, CAS = Chemical abstract service, Ref = Reference, Qual = Qualitative.

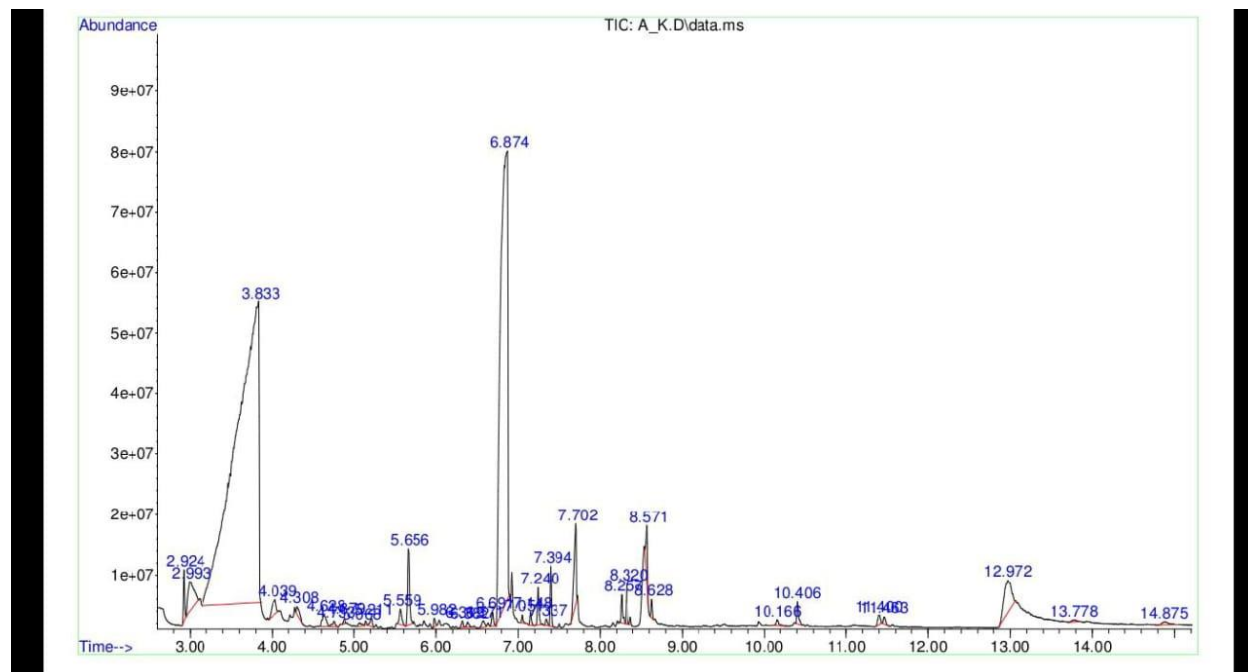


Fig. 1: Total ion Chromatogram of ethanol extract of *Cinnamomum tamala* leaf extract

3.3 DPPH scavenging activity of *Cinnamomum tamala* leaf extract

The results of the antioxidant activity expressed as inhibition (%) of DPPH radical of *C. tamala* leaf extract are shown in Table 2. This was demonstrated by the extract's ability to act as hydrogen atoms or electron donors in the conversion of the stable purple-coloured DPPH to the reduced colourless DPP-H.

The results indicated the scavenging ability of the leaf extract was concentration-dependent. The reducing capacity of the extract, another significant indicator of antioxidant activity was

also found to be appreciable and comparable with ascorbic acid, which was used as standard. Muchuweti *et al.*, (2006) reported that the measured antioxidant activity of plant extracts may be due to the synergistic effect of polyphenolics with one another and/or with other components present in an extract. The high antioxidant activity of *C. tamala* leaf extracts may be attributed to their high profile of oxygenated compounds, especially hydroxyl groups since the increase in activity is dependent on the donation of hydrogen from the extract to form the DPPH-H.



Table 2: DPPH radical scavenging activity of *C. tamala*

Concentration (µg/L)	% Inhibition of <i>C. tamala</i>	% Inhibition of L-ascorbic acid
150	73.30	73.74
250	73.14	78.52
350	76.80	83.29
450	78.90	90.45
550	79.20	95.22
650	80.10	98.32
750	80.60	98.80
850	91.40	99.28
950	93.50	99.76

3.4 Ferric reducing property of *Cinnamomum tamala* leaf extract

FRAP method compares antioxidants based on the ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions through the donation of an electron with the resulting ferrous ion. The results in

Table 3 revealed that the values of reducing the power of the plant extract were directly proportional to its concentration. The reducing capacity of extracts is much related to the presence of biologically active compounds (phenols) with potent donating abilities (Li *et al.* 2006).

Table 3: Ferric reducing Activity of *C. tamala*

Volume (g/ml)	Ethanollic extract (Absorbance)	FeSO_4 (Absorbance)
0.10	0.218	0.006
0.40	0.226	0.017
0.80	0.262	0.014
1.00	0.287	0.049
1.12	0.312	0.043
1.50	0.342	0.103

3.5 Antimicrobial assay of *C. tamala* extract

The antimicrobial activity of *C. tamala* was carried out using the agar diffusion method. The antimicrobial activity (as depicted by the

clear zone inhibition) of *C. tamala* leaf extract at different concentrations (20 -100mg/ml) is presented in Table 4. A total of 10 microorganisms consisting of three (3) Gram-positive, five (5) Gram-negative bacteria and two (2) fungi were assayed. Standard antibiotics and antifungal drugs (Gentamycin and Nystatin) were used as a control for bacterial and antifungal respectively.



The result obtained in this study showed that the microorganisms were susceptible at different concentrations of the extract, with the highest activity recorded at the highest concentration.

The most susceptible bacteria at 100mg/ml were *Enterobacter sp* (25mm), *Shigella sp* (25mm), *Pseudomonas aeruginosa* (23mm) *Bacillus subtilis* (20mm), *Staphylococcus aureus* (16mm) and *Staphylococcus epidermidis* (18mm). The bacteria strains. *coli* and *Salmonella sp* were resistant to the effect of *C. tamala* extract as no activity was recorded at different concentrations. On comparing the efficacy of the extract with that of antibiotic gentamycin, which was used as a control, it was observed that the zone of inhibition of these extracts was moderate compared to that of the

drug. The antifungal activity of the extract of *C tamala* against the various strains of fungi such as *Aspergillus. Niger* and *Candida albicans* used in this study revealed that the extract inhibited the growth of the fungi at different concentrations. According to Junior and Zani (2000), the diameter of the inhibition zone: <9 mm is inactive; 9-12 mm, is partially active; 13-18 mm, is active; >18 mm, is very active.

Generally, the antimicrobial activity of the extract of *C tamala* revealed that gram-positive and gram-negative bacteria as well as fungi were susceptible to this extract, indicating that *C. tamala* may be used as a broad-spectrum antimicrobial agent. This further corroborates its use in the treatment of various ailments.

Table 4: Antimicrobial assay of *C. tamala* extract

Test organisms	Extract concentration(mg/ml)/zones of inhibition diameter(mm)					
Concentration	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml	Control
<i>Staphylococcus aureus</i>	-	-	8	13	16	32mm
<i>Bacillus subtilis</i>	6	8	12	16	20	15mm
<i>Staphylococcus epidermis</i>	-	8	11	14	13	37mm
<i>Salmonella sp</i>	-	-	-	-	-	18mm
<i>Shigella sp</i>	13	17	18	21	25	16mm
<i>Escherichia coli</i>	-	-	-	-	-	36mm
<i>Pseudomonas aeruginosa</i>	8	12	15	18	23	41mm
<i>Enterobacter sp</i>	12	16	18	21	25	33mm
<i>Candida albicans</i>	-	-	13	20	24	
<i>Aspergillusniger</i>	10	12	14	17	21	

4.0 Conclusion

The ethanol extract of bay leaf obtained in this study was evaluated for its bioactive compounds, antioxidant and antimicrobial

activities. The results revealed the extract contained moderate to significant concentration of important phytochemical compounds. The extract of *C. tamala* exhibited strong reducing and free radical scavenging



properties when compared with standard compounds, which may be due to the presence of polyphenols and other bioactive compounds. The antibacterial activity of the extract against tested bacteria and fungi strains shows that it has the potential to be used as broad spectrum antibiotics for the treatment of various ailments, thereby validating the ethno-pharmacological importance of bay leaf.

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