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Uncovering the Antimicrobial Potential of Different Polarities of Components Extracted from *Nauclea latifolia* Leaves

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Abstract

Antibiotics formerly effective against many infections are worthless since certain bacteria have become immune to them. The nanoparticles were likened to the extracts' actions. Grinding plant materials were mixed with copper oxide and suspended in ethanol and distilled water to form plant nanoparticles, and they were subsequently filtered to get crude extracts (CuO). This article aims to isolate the antibacterial activity-rich fractions from a 50 percent methanol extract of dried *Nauclea latifolia* leaf. Using preparative thin layer chromatography, we separated constituents from a water solution of a 50 percent methanol extract of dried *Nauclea latifolia* leaf by first partitioning it with hexane, then ethyl acetate (EA), and finally butanol, yielding fractions of varying polarity. The findings demonstrate that the extract, fractions, and elements may be used to create phytomedicinal formulations for treating bacterial and fungal illnesses.

Keywords: Antimicrobial activity, *Nauclea latifolia*, chromatography, solvent polarity, and aqueous Methanolic extract.

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1. Introduction

Nauclea latifolia may be either a multi-stemmed shrub or a small tree, which can live for a very long time. The barks and leaves are used to make infusions and decoctions that are then employed to treat various ailments, including tropical illnesses like infectious diseases, high temperature, diarrhea, and stomach problems, in West and South Africa. *Nauclea latifolia* is a popular chew stick and medicine in Kano (Nigeria) for the treatment of TB and discomfort in the stomach [1]. The multi-use plant *Nauclea latifolia* provides substantial amounts of forage in moist and dry weather. Protein and calories are comparatively rich in *Nauclea latifolia* leaf, and it also has a lot of other nutrients, including iron, calcium, zinc, potassium, vitamin C, and vitamin A. In tiny ruminant feeding systems, the forage's worth and advantages as a high-quality supplementary to roughages of poor quality have not been fully studied or utilized [2]. The animals like it in fresh and evaporated forms, and it has an extensive record of therapeutic, pharmacological, and ethno-veterinary applications. Anti-ulcer, antibacterial, antihelminthic, antiplasmodial, antiviral, antitrypanosomal, antioxidant, hypolipidemic, neuropharmacological, antidiabetic, hypoglycemic, histomorphological, wound healing, anti-inflammatory, hepatoprotective, hypocholesterolemic, hepatotoxic, and nephrotoxic effects have all been observed in studies of *Nauclea latifolia* [3]. This plant contains a variety of phytoconstituents, including alkaloids, tannins, tanning compounds, and saponins. Extracts of the roots, leaves, and stem barks are used in antimalarial and eczema medicines in their raw form. *Ocimum gratissium* L., commonly called "Clove basil" and "African basil," is a polymorphic, branching, fragrant shrub that may grow anywhere from half a meter to three meters in height. It is a member of the plant group Lamiaceae [4]. The plant's natural range includes most of southern Asia, much of Africa, and a few spots in South America. *Ocimum gratissium* has been shown to have antibacterial, diabetic, diarrheic, mutagen, and anticancer properties. It has been scientifically demonstrated that *Ocimum gratissium* has a variety of active elements which are good for one's health. The additional metabolite of a plant is what determines its effect on complicated formation. In several works, authors detail how they used plant extracts to make nanoparticles. These synergies frequently increase antimicrobial efficacy towards a wider range of pathogens [5]. Strong antioxidant and modest antibacterial activity were found in *N. latifolia* extracts and isolated components. Therefore, these agents may be useful in treating illnesses caused by oxidative stress or infection. These results are backed by the traditional medical practice of using *N. latifolia* extracts [6]. To employ solvents with varying degrees of polarity to extract active compounds from three plants with a history of usage in the treatment of malaria. Extraction was done using EA, n-hexane, water, and dichloromethane: methanol (1:1) on the leaf of Tithonia

diversifolia, Lawsoniainermis, and *Nauclea latifolia* [7]. Mice infected with Plasmodium berghei were 24 overall and were split into eight teams of three. Every one of the teams (A-F) received either 100, 300, or 500 mg/kg of an extract. Negative control (group G) was given 2 mL of normal saline, whereas positive control (group H) was given five mg/kg of chloroquine. Oral dosing with the medications and extracts occurred one time per day for a total of five days [8]. To assess the phytochemical components, antibacterial, antioxidant, and anti-diabetic properties of *Nauclea latifolia* Smith whole fruit extracts. Using the micro-broth dilution technique, the antibacterial potential was assessed. Over two hours, the degree of mortality was studied against representative species Shigella sonnei and Staphylococcus aureus at various concentrations [9]. Although the extract's inhibitory effectiveness against -glucosidase and -amylase was only marginally strong, it might be employed at large concentrations to prevent the negative effects of synthetic inhibitors. The creation of indigenous, low-cost medicines with natural origins is urgently needed due to the rising prevalence of diabetes in urban and rural populations worldwide [10]. They essentially supported the phytochemical components and anti-plasmodial effectiveness of bioactive phytochemicals embedded in Naucleadiderrichii, validating the plant's traditional usage as a treatment for malaria and other clinically relevant disorders [11]. This research concludes that lead acetate-induced toxic rats may recover from hematological abnormalities and lung histological damage after receiving methanol [12].

There are terpenes, alkaloids, and glycoalkaloids in *Nauclea latifolia*. Therapy of skin problems using leaves is quite effective in South-Western Nigeria. The goal of this research was to examine the antibacterial action of several leaf extracts, including a 50 percent methanol extract, a non-polar fraction generated by successive extraction of the water solution of the 50 percent methanol extract, and a polar fraction, with EA, hexane and butanol, and the elements separated from the fractions using “Thin layer chromatography (TLC)”, to evaluate the plant's purported usefulness in treating a variety of skin ailments.

2. Materials and methods

2.1 Sampling, recognizing, and processing of plants

N. latifolia leaves were acquired in bulk from June to August 2007 in the Mushin and Oyingbo marketplaces in Lagos, Nigeria. Dr. A.A. Adekunle of the University of Lagos's Division of Botany and Microbiology and Mr. Felix Usang of the “Forestry Research Institute of Nigeria (FRIN)”, Ibadan, successfully recognized them and submitted a voucher to the Herbarium of the Botany Division (Figure 1). The new leaf was broken up and dried in the air for three weeks in a free of dirt room temperature environment before being pulverized using a mixer with metal blades. Experiments typically yielded 2.65 kg of fresh leaves, and we could dry 1.12 kg of material. Seventy-two hours were spent extracting 400 g of leaf powder in 3 L of Occasionally using a 50 percent methanol solution in room temperature water, gently stirring. After filtering, 500 mL of the solvent was used to extract the leftover residue for another 24 hours. In a vacuum oven at 55 °C, the sum of the filtrate was concentrated to provide after freeze-drying. The brownish substance measured 75 grams and became a thick gel. This is an extract made out of 50% methanol. A prior study outlined the steps to dissolve it in 50 ml of distilled water and then extract it progressively. The fractions of orange butanol (1.08 g), brown EA (1.02 g), and greenish-yellow hexane (57 mg) were collected following the evaporation of the solvent from using 6 x 10 cm hexane, 9 x 10 cm EA, and 7 x 10 cm butanol.

Figure 1: Herbarium Samples

2.2 Element isolation via Thin Layer Chromatography (TLC)

Chromatographing tiny amounts of every fraction on gel-coated aluminum plates helped find the best solvent combination for the preparative TLC. The dichloromethane-hexane-methanol (50:40:10) solvent. The mixture was used to isolate the hexane fraction, which was then split into two parts. These parts were designated Hex a and Hex b. Employing a solvent combination of EA, methanol, water, and hexane, the EA fraction was divided into four components labeled EA- a, b, c, and d. Employing a solvent combination consisting of EA, methanol, water, and dichloromethane, the butanol fraction was divided into five parts, which were given the labels But-a, b, c, d, and e. Silica gel was transmitted out on glass plates, and then the different fractions were put to it and fractionated using the appropriate solvent systems. The bands were examined under ultraviolet light to determine their composition, and then scraped into fresh beakers where the components were dissolved in a solvent combination of methanol and chloroform (1:1). Hex a and Hex b were obtained from 57 mg of the hexane fraction in a typical the test. EA a, EA b, EA e, and EA d were all extracted from 250 mg of EA fraction. Two hundred milligrams of the butanol fraction produced 12 milligrams of But a (deep orange), 12 milligrams of But b (light orange), 11 milligrams of But e (light yellow), 10 milligrams of But d (light orange), and 10 milligrams of But e (light yellow).

2.3 *Preparation of a 50% methanol extract, fractions, and elements for antimicrobial testing*

The hexane fraction, the hexane components Hex a and Hex b dissolved in hexane, the EA fraction, and the components EA a, EA b, EA c, and 50 percent methanol extract “dissolved in dimethyl sulphoxide (DMSO)” were the solutions used for the evaluation of the antimicrobial

activity. The standard was a suspension of 0.05 percent of proloxacin. Antimicrobial efficacy was determined for every one of the neat solvents that were employed.

2.4 *Evaluation of antimicrobial efficacy*

The microorganisms used in the experiment were collected from the samples located at the Division of Medical Microbiology and Parasitology in the College of Medicine. They are namely, "Bacillus subtilis, Citrobacter trendi, Enterobacter faecalis, Escherichia coli ATCC 25922, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus albus, Staphylococcus aureus ATCC 25923, Staphylococcus aureus and Candida albicans". The test was done using the same methodology used in previous tests of extracts obtained from several medicinal plants. The evaluation was conducted using a volume of fifty microliters containing the test ingredients. Positive antimicrobial action was evaluated based on the presence of distinct regions of inhibition that were determined in millimeters and located within the water sources.

3. Results and Discussions

Table 1 displays the production and R values of the components, as well as the masses of the fractions utilized for the preparative TLC, also the colors of the elements and fractions. Components recovered from a 50 percent methanol extract of dried *Nauclea latifolia* leaf are shown in figure 2 with their corresponding Rf values. The outcomes of the sensitivity analyses are shown in Table 2 and Table 3. Hexane, EA, and dimethyl sulfoxide (DMSO) were employed to dissolve the extracts and components, and none of them were harmful to the bacteria's ability to multiply. The 50% methanol extract showed strong activity toward "*E. coli* ATCC 25922, B. subtilis, *P. mirabilis*, *E. coli*, P. aeruginosa, *S. aureus* ATCC 25923, and *S. aureus*", but only inadequate action towards "*E. faecalis* and no activity against *C. trendi*, K. pneumoniae, *S. albus*, and C. albicans", which were the organisms used as controls. The fact that its effectiveness towards P. aeruginosa was higher than the control was very important. The butanol fraction's antimicrobial test results are shown in figure 3.

The butanol fraction was very effective against the bacteria Escherichia coli ATCC 25922 and Proteus mirabilis but only moderately effective toward Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, and Candida albicans (11-12 mm inhibitory zone). The element, while showing significant action against *P. mirabilis*, it did not affect any of the other experimental species. The element But, b did not affect most of the bacteria and yeasts that were examined. The element But it was very effective toward *S. albus*, and it was only moderately effective toward *P. mirabilis* and P. aeruginosa and completely ineffective toward the other nine bacteria tested. Element But d showed high action towards C. albicans, with an inhibitory zone of 14 mm. Still, poor activity towards B. subtilis and *E. coli* ATCC 25922, and did not act towards the other nine bacteria. While it showed action comparable to the command towards P. aeruginosa, it was much less effective for *E. coli* and *P. mirabilis*. It had zero impact on the other pathogens. The results of antimicrobial tests on different fractions and components are shown in figure 4.

Inhibitory zone measurements of 16, 15, and 14 millimeters were recorded for B. subtilis, K. pneumoniae, and P. aeruginosa, respectively. The EA fraction showed less action toward "*E. coli* ATCC 25922, *P. mirabilis*, and C. albicans" and no action against the other six bacteria. Constituent EA, to a greater or lesser extent than the control, EA significantly inhibited the growth of Escherichia coli and Proteus mirabilis but did not affect the other bacteria. Therefore, EA a may be sufficient for treating an *E. coli* infection, eliminating the need for the 50 percent methanol extract. Constituent EA. b showed significant efficacy toward "*C. trendi*, *S. albus*, and *S. aureus* ATCC 25923", alost on par with the control. There was no evidence of efficacy toward these three bacteria in the EA fraction. Component. EA. b was also potent toward B. subtilis, *S. aureus*, and C. albicans, but to a lesser extent. Therefore, component EA b may be all needed to treat infections caused by "*C. trendi*, *S. albus*, *S. aureus* ATCC 25923, and Calbicans". Moreover, it did not affect the development of the other six bacteria. Constituent EA e showed considerable efficacy, on the same level as the management, toward *S. albus*, but only moderate action toward "B. subtilis, *C. trendi*, *S. aureus*, and C. albicans", and that there is no development inhibition toward the other seven species. The Role of EA to identical amount as elements EA b and EA e, EA d was exclusively efficient toward C. albicans; it did not affect the development of the other eleven bacteria tested. Notably, the EA fraction and its constituents lacked anti-*E. faecalis* activity. The hexane fraction showed activity toward Escherichia coli (13 mm inhibitory zone) and Candida albicans (less performance) but did not affect the other eight bacteria tested. Consisting of hexane, the 15 mm inhibitory region demonstrated that Hex a was very active toward C. albicans, whereas the hexane component was less effective toward *S. aureus*. Hex b showed the strongest activity against C. albicans out of all the tested extracts, fractions, and elements but the lowest effectiveness toward *S. aureus*. These two bacteria were the only ones affected by the two hexane elements. Bacillus subtilis, Enterococcus faecalis, Escherichia coli ATCC 25922, Pseudomonas aeruginosa, Pseudomonas mirabilis, and *S. aureus* were all killed by the 50% methanol extract, and all but *E. faecalis* showed the antimicrobial effects on the same level with ciprofloxacin. While the 50% methanol extract showed effectiveness toward all eight of the bacteria mentioned above, the butanol fraction was only effective towards 7, the EA fraction only toward 6, and the hexane fraction only toward 2. Moreover, a closer look at the data reveals that the But fraction was effective towards K. pneumoniae and *S. albus*, while the EA and hex fractions were effective toward C. albicans. The extract prepared with 50% methanol showed no antimicrobial activity. Since the three produced fractions, if taken together, showed antibacterial effects toward a wider range of microorganisms, fractionating the 50% methanol extract into these three was very beneficial. According to the findings, the butanol fraction exhibited greater effectiveness than any of its elements against "B. subtilis, *E. coli* ATCC 25922, and both K. pneumoniae and *S. aureus* 25923" toward none of the elements was working.

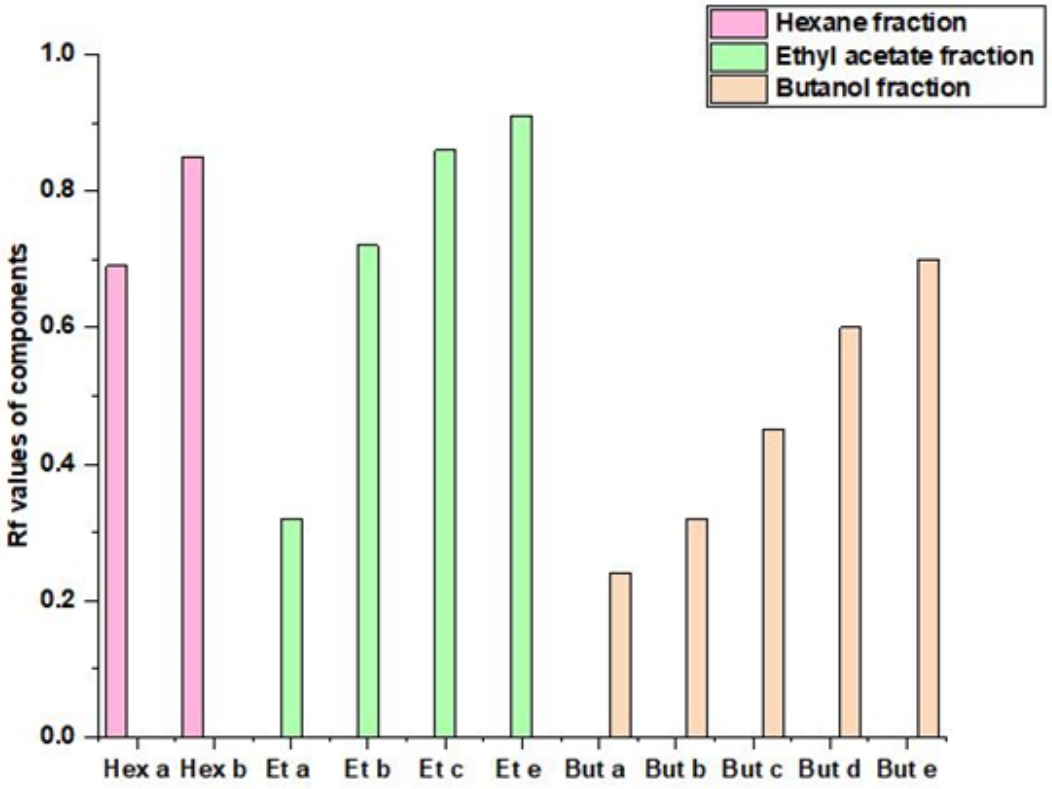

Figure 2: Rf values of components

Table 1: *Nauclea latifolia* leaf extracts in 50% methanol yield various fractions and components

Fraction	Mass fractionated (mg)	Colors of fractions	The solvent system used for fractionation	R_f values of components	Color components	Masses of members (Mg)
Hexane fraction	57	Faint greenish Yellow	Dichloromethane Hexane-methanol 50:40:10	Hex a 0.69	Colourless	19
				Hex b 0.85	Colourless	18
EA fraction	250	Brown	EA -methanol-water-home 70:15:10:1	EA a 0.32	Light brown	13
				EA b 0.72	Deep brown	12
				EA c 0.86	Deep brown	30
				EA d 0.91	Brown	20
Butanol fraction	200	Orange	EA -methanol Water dichloromethane 7:2:1:1	But a 0.24	Deep orange	12
				But b 0.32	Light orange	12
				But c 0.45	Light yellow	11
				But d 0.60	Light orange	10
				But e 0.70	Light yellow	10

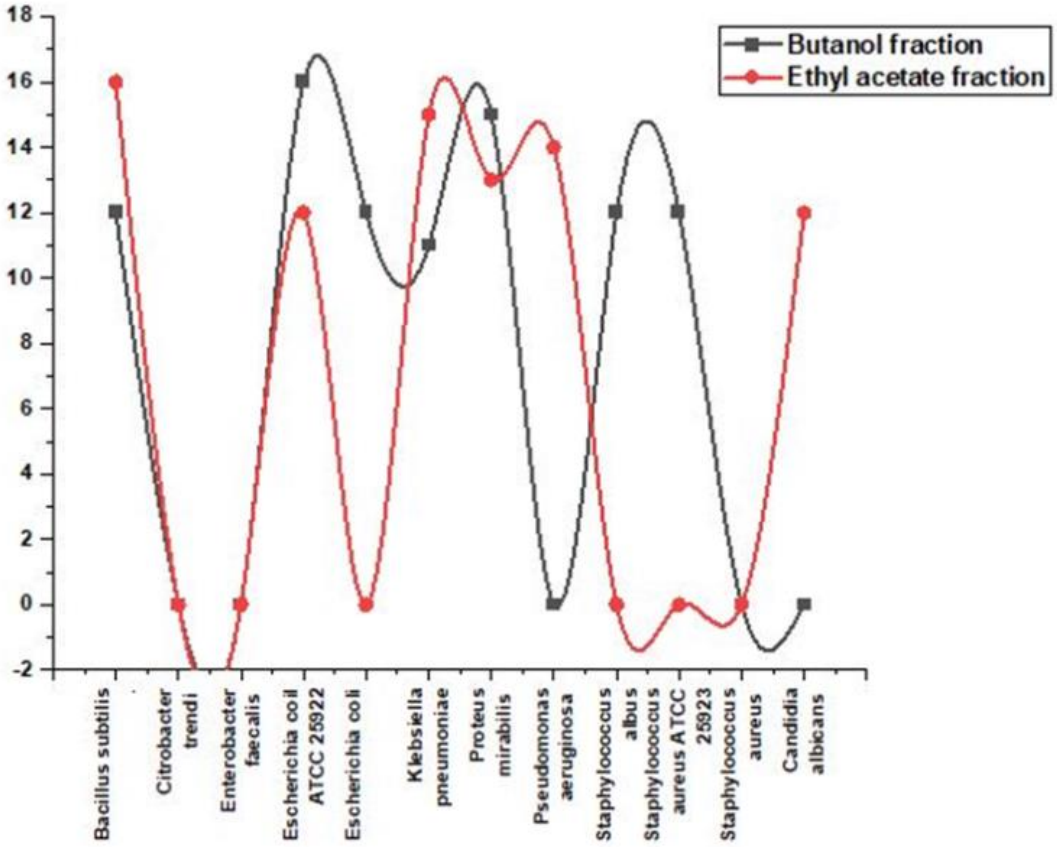

Figure 3: Butanol fraction's antimicrobial test

Table 2: Butanol fraction's antimicrobial test obtained from the *Nauclea latifolia* leaf extracts in 50% methanol

Microorganisms	Butanol fraction	EA fraction	Butanol Comp. a	Butanol Comp. b	Butanol Comp. c	Butanol Comp. d	Butanol Comp. e	50%methanol extract
Bacillus subtilis	12	16	-	-	-	10	-	12
Klebsiella pneumoniae	11	15	-	-	-	-	-	-
Enterobacter faecalis	-	-	-	-	-	-	-	7
Escherichia coli ATCC 25922	16	12	-	-	-	12	-	20
Escherichia coli	12	-	-	-	-	-	14	20
Staphylococcus aureus ATCC 25923	12	-	-	-	-	-	-	25
Pseudomonas aeruginosa	-	14	-	-	16	-	20	25
Proteus mirabilis	15	13	20	-	11	-	12	21
Staphylococcus albus	12	-	-	-	20	-	-	-
Candidia albicans	-	12	-	-	-	14	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	25
Citrobacter trendi	-	-	-	-	-	-	-	-

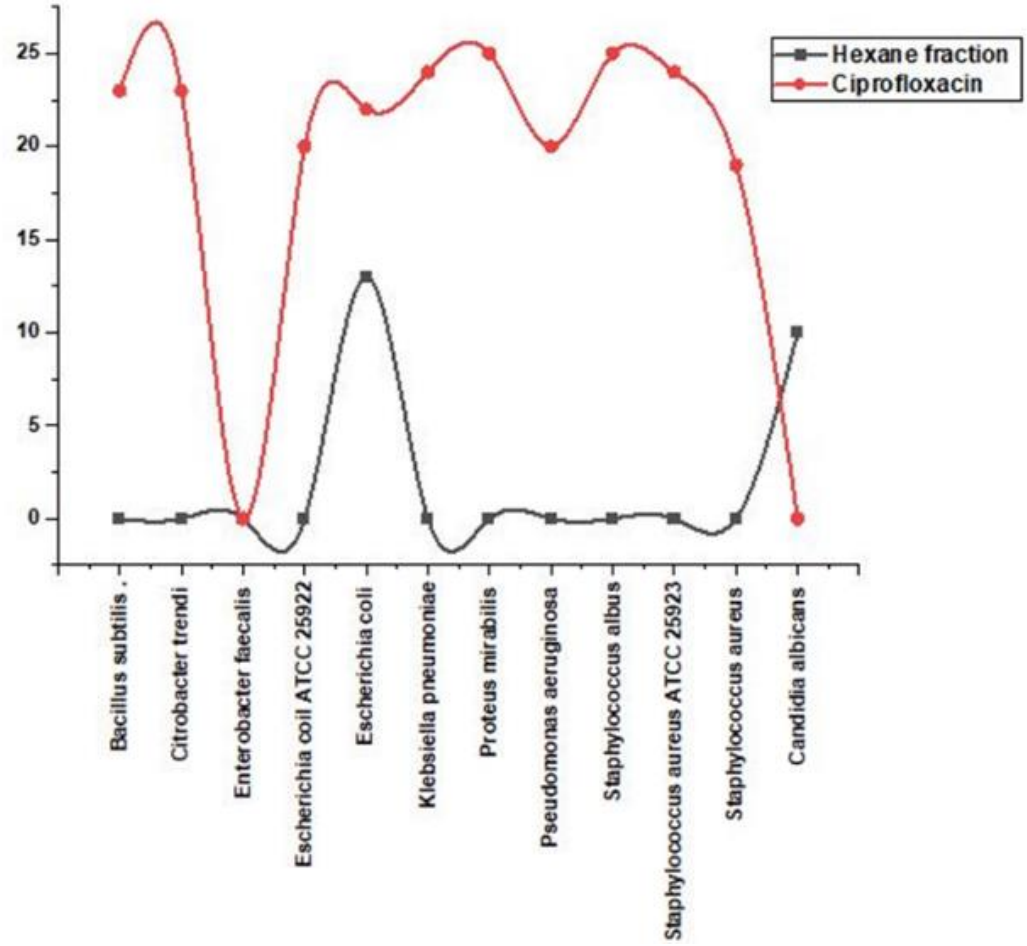

Figure 4: Outcome of the Hex, EA, and Ciprofloxacin fractions

Table 3: Outcome of the Hex, EA, and Ciprofloxacin fractions

Microorganisms	EA Comp. a	EA Comp. b	EA Comp. c	EA Comp. d	Hexane fraction	Hexane Comp. a	Hexane Comp. b	Ciprofloxacin
Bacillus subtilis	-	10	16	-	-	-	-	23
Klebsiella pneumoniae	-	-	-	-	-	-	-	24
Escherichia coli	22	-	-	-	13	-	-	22
Escherichia coli ATCC 25922	-	-	-	-	-	-	-	20
Enterobacter faecalis	-	-	-	-	-	-	-	-
Proteus mirabilis	12	-	-	-	-	-	-	25
Candidia albicans	-	15	14	15	10	15	17	-
Staphylococcus aureus ATCC 25923	-	22	-	-	-	-	-	24
Staphylococcus albus	-	25	22	-	-	-	-	25
Citrobacter trendi	-	23	14	-	-	-	-	23
Staphylococcus aureus	-	13	14	-	-	11	10	19
Pseudomonas aeruginosa	-	-	-	-	-	-	-	20

On the other hand, the fraction displayed less activity toward *E. coli, P. mirabilis,* and *S. albus*. But had no action toward *C. trendi*, *E. faecalis*, and *S. aureus*. But, performance toward just one bacterium, *P. mirabilis*, was seen when butanol was combined with a, and this action was much higher than that of the But fraction and the other butanol constituents. But, b showed no signs of activity toward any of the tested bacteria. But c was extremely aggressive toward *S. albus*, and its activity level was comparable to that of the management. But e, But d, and But e were very effective in inhibiting the growth of three different bacteria. But whereas the But fraction and the other But elements were inactive towards C. albicans, d was effective toward the fungus. Therefore, element But d will be useful in treating illnesses brought by C. albicans. To determine which aspects of the butanol fraction are effective against a certain microorganism, it is necessary first to fractionate the butanol fraction into its constituent parts. For instance, the elements But e and But e exhibited high levels of activity in P. aeruginosa, making it a more effective treatment than the butanol fraction in cases when the illness is caused by P. aeruginosa. It is important to note, moreover, that although the But fraction displayed antibacterial activity toward *S. aureus* ATCC 29523, none of the elements displayed the same level of effectiveness.

Both the bark and leaf methanol extracts were very active toward 6 different microorganisms: "Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Candida albicans, and Aspergillus niger". The petroleum ether extract of the leaf showed antimicrobial activity toward Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and Candida albicans; bark extract was effective against all five bacteria plus P. aeruginosa. The research found that although all of the microorganisms tested were significantly inhibited by the methanolic extracts of the leaves and bark, but not by the petroleum ether extracts [13]. Pathogenic bacteria such as Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, and Shigella dysenteriae were examined using water and ethanolic extracts of the leaf, bark, and roots. Leaf extracts in both water and alcohol showed modest antimicrobial activity. Two leaf extracts were used to test the bacteria's susceptibility; the results showed that K. pneumoniae and *S. aureus* were equally susceptible, whereas P. aeruginosa and *E. coli* were similar but less susceptible. When compared to the leaf extracts, the antimicrobial action of the water and ethanolic root extracts was significantly reduced [14]. The researchers tested the antibacterial effects of *N. latifolia* extracts on four different clinical bacterial isolates: *S. aureus*, *E. coli*, Salmonella typhi, and Pseudomonas aeruginosa, chloroform, diether, and petroleum ether. Not even root and leaf extracts could stop S. typhi from spreading. To a greater extent than *S. aureus*, P. aeruginosa was killed by the different leaf extracts [15].

4. Conclusions

The 50% water methanol extract and numerous fractions and components produced from the leaf of *N. latifolia* strongly suppress the growth of several different microorganisms, including "Gram-positive and Gram-negative bacteria," also fungal organisms. The outcome suggests that, although having just a low level of activity against *E. faecalis*, it may still be possible to create a medication that is successful for the therapy of infections brought through all of the pathogens described, provided that the necessary decisions are made about the extract, fractions, and ingredients.

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