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*Original Research Article*

# Inhibition of Dehydrogenase Activity in Sputum Bacterial Isolates by hot aqueous extracts of selected Nigeria anti-cough plants

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ABSTRACT

The exploration of plant-based medications as primary therapeutic agents for the treatment of various ailments is currently ongoing. The present study evaluates the antimicrobial properties of hot aqueous leaf extracts of *Piper guineense, Gongronema latifolium*, and *Vernonia amygdalina* against clinical sputum isolates. The isolates were characterised based on morphology and biochemical properties. The dehydrogenase enzyme assay and the Agar-well diffusion techniques were used in evaluating the antimicrobial activity of plants, singly and in combination at ratios of 1:1:1. *Streptococcus species* (46.16%), *Klebsiella species* (23.08%), *Escherichia coli* (15.38%), and *Proteus species* (15.38%) were all present in the bacteria isolates at high prevalence rates. The aqueous extract of *V. amygdalina* produced the highest level of antimicrobial activity, followed by the combined extract, which inhibited the growth of *Klebsiella spp* and *Escherichia coli* at 200 mg/mL. At 200 mg/mL, *G. latifolium* and *P. guineense* inhibited *Streptococcus species* with a 14 mm-diameter zone. When compared to the standard drug, Gentamicin, results of the dehydrogenase activity assay indicate that *V. amygdalina* and the combined extract exhibited the most favourable $IC_{50}$ values of 8.69 g/mL, and 9.04 g/mL, 6.37 g/mL, and 9.87 g/mL against *E. coli* and *Proteus spp*, respectively. The high $IC_{50}$ values indicated that *Klebsiella spp* resisted the plant extracts considerably. Plant aqueous extracts have shown that they are potential natural drug agents for treating and managing cough-causing organisms.

***Keywords:*** antimicrobial activity, dehydrogenase Assay, *Vernonia amygdalina*, *Piper guineense, Gongronema latifolium*, bacteria isolates, cough.

## Introduction

Organic plant materials are the source of the majority of synthetic medicine.[1] Plant products are incorporated into various substances such as modern pharmaceuticals, dietary supplements, and folk medicines for synthetic drugs. [2] Several phytomedicines are utilised to treat pathogen-induced ailments. Pathogenic bacteria, which can rapidly spread in the body and replace the normal flora in normally sterile tissues, can cause infections and disease.[3] Underdeveloped and developing nations use the extracts and infusions of medicinal plants and plant products to treat and manage diseases. [4] Current antimicrobial agents are losing their potency due to the recurring emergence and re-emergence of organisms resistant to antibiotics, a persistent concern in microbiology. Observations have shown that common antibiotics cause pathogenic organisms to undergo evolutionary modifications, exacerbating the problem.[5] Assessments of cellular viability and cytotoxicity commonly employ dehydrogenase-dependent assays due to their ability to document cellular conditions. Antimicrobial assays that utilise recording mechanisms are considered safer, more user-friendly, and exhibit greater reproducibility than alternative methods.

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The ability of an antimicrobial medication to prevent pathogenic bacteria infection depends critically on its ability to inhibit total microbial dehydrogenase enzyme activity.[6] This method allows for the measurement of bacterial metabolism and growth. The activity of the dehydrogenase enzyme directly correlates with the number of current active cells.[7] Coughing, whether voluntary or involuntary, is a natural defence mechanism that helps remove foreign objects, bacteria, irritants, fluids, and mucus from the respiratory system. More than 200 different bacterial species live in the upper respiratory tract.[9] These microorganisms cause a variety of respiratory illnesses in humans. Most individuals are habituated to utilising modern medications to treat and control coughs. However, repeated use of these pharmaceuticals can lead to drug dependence and antibiotic resistance. Throughout history, plants have been utilised to treat various respiratory illnesses associated with coughs, such as influenza, the common cold, and pneumonia. Plants contain phytocompounds with various properties, such as antibacterial, antifungal, antioxidant, expectorant, antitussive, and demulcent, that aid in the body's defence against cough-causing pathogens. [10] *Vernonia amygdalina*, from the Asteraceae family, is a small shrub with elliptic-shaped, petiolate leaves approximately 6 mm in diameter. Due to its bitter taste, people commonly refer to it as a "bitter leaf," a plant that thrives in tropical climates, particularly in Nigeria, Cameroon, and Zimbabwe, among other regions of Africa. Various names for this plant exist, such as "Ewuro" in Yoruba, "Onugbu" in Igbo, "Oriwo" in Bini, "Ityuna" in Tiv, "Chusar Doki or fatefate" in Hausa, and "Etidot" in Cross River State. The leaves are a distinct variety of verdant foliage that can be consumed either raw or cooked and are classified as a leafy green vegetable. There are numerous health benefits associated with it. It exhibits efficacy in combating digestive ailments such as amoebic dysentery and possesses antimicrobial and antiparasitic properties. [11,12] The leaves can be heated

to a high temperature or soaked in several changes of water to reduce the plant's high saponin and acid content. [13] Aqueous preparations of the leaves as tonics can be used to treat various ailments. Numerous African herbalists and traditional healers recommend aqueous extracts derived from indigenous plants to address various medical conditions, such as diabetes mellitus, emesis, nausea, appetite loss, dysentery, and gastrointestinal tract disorders. Researchers have found the leaves to be effective in treating fever due to the availability of a quinine substitute. [14]*Piper guineense* is classified as a perennial botanical specimen within the Piperaceae family and the Piper genus. The plant is a spice native to West Africa known by various names such as climbing black pepper, West African black pepper, Ashanti pepper, Guinea cubeb, and Benin pepper. In Nigeria, called *Uziza* in Igbo, *Iyere* in Yoruba, the leaves and seeds are used as spices, food preservatives, insecticides, herbal medications, and a fragrance in the cosmetic industry. [15] It also treats arthritis, bronchitis, diarrhoea, and cough. [16] It protects against oxidative stress and inflammation, improves the activity of digestive enzymes, and lowers lipid peroxidation.[17] Its biological impacts include insecticidal, antimicrobial, antifungal, and antioxidant advantages.[18] *Gongronema latifolium* is a plant species with a broad leaf, taxonomically classified within the Asclepiadaceae family. *Gongronema latifolium* is found in the equatorial rainforests of West African nations like Nigeria, Ghana, Côte d'Ivoire, Sierra Leone, and Senegal. The plant known as 'Utazi' in Igbo and 'Arokeke' in Yoruba is a prevalent crop in the southern region of Nigeria. Research has shown that the whole plant has many different herbal therapeutic effects. These include antimicrobial activities against bacteria, fungi, parasites, and viruses; digestive tonicity; antipyretic effects; antioxidant actions; hepatoprotective potential; antitumor activities; anti-inflammatory effects; antiulcer properties; anti-sickling capabilities; mild expectorant effects; analgesic effects; hypoglycemic and hypolipidemic effects; and laxative effects. [19] When consumed raw or infused with hot water, the leaves exhibit medicinal properties that can effectively alleviate symptoms such as runny nose, catarrh, nasal congestion, and cough.[20] According to reports, *G. latifolium* is utilised as a respiratory remedy in Nigeria. [21] Asthmatics can chew on fresh *G. latifolium* leaves to lessen their wheezing, and they can ingest the plant's roots by cold maceration. [22]This study investigates the antimicrobial properties of hot aqueous leaf extracts of *P. guineense, G. latifolium,* and *V. amygdalina* against cough-causing microorganisms. These plants are commonly used in traditional medicine to treat coughs. The cellular dehydrogenase enzyme and antimicrobial activity studies were used as assays to determine the effectiveness of the plants.

## Materials and Methods

*Plant Materials*

Fresh leaves of *P. guineense, G. latifolium,* and *V. amygdalina* were purchased from the Relief Market, a well-known marketplace in the Owerri Municipal Local Government Area of Imo State, in October 2022. They were subsequently identified by Mr Francis Iwueze, a plant taxonomist affiliated with the Department of Wildlife and Forestry at the Federal University of Technology Owerri (FUTO), located in Imo State with voucher numbers FUTO/FWT/ERB/2022/88, FUTO/FWT/ERB/2022/89 and FUTO/FWT/ERB/2022/90, respectively

*Plant sample preparation*

The plants were spread out and subjected to two (2) weeks of air-drying at room temperature. The plant samples were pulverised into a fine particulate form using an industrial-grade grinding machine and placed into distinctly labelled containers for easy identification and storage. Standardised preparation methods were employed for the aqueous extraction of samples. About 450 g of ground plant samples were dissolved in a 2.5-litre volumetric flask containing distilled water that was heated to a boiling point. The solution was then brought to the desired volume and boiled for 30 minutes. Subsequently, the solution was decanted, filtered, and freeze-dried. The samples were stored in appropriately labelled containers and maintained under refrigeration at 4°C, protected from light and moisture.

*Preparation of the media*

Nutrient agar powder (12 g) was dissolved in 500 mL of distilled. Samples were thoroughly mixed before autoclaving for 15 minutes at 121°C. After cooling, the mixture was aseptically transferred into 18 petri dishes. The blood sample was aseptically introduced and thoroughly mixed at room temperature; 15 mL was dispensed under aseptic conditions into Petri dishes that had been sterilised beforehand. The substance was allowed to solidify under ambient conditions, subsequently labelled, and stored at 4 °C in an airtight plastic container to mitigate any potential moisture. Nutrient broth was prepared in a conical flask by carefully blending 1L of distilled water and 13 g of nutritional broth powder. The mixture was then autoclaved for 15 minutes at 121°C.

*Sputum sample collection and preparation*

Twenty-two (22) fresh sputum samples were obtained from willing patients at the Federal Medical Centre, Owerri, Imo State (FMC) using sterile containers. The blood agar was streaked with the sputum samples and kept for 24 hours at 37 °C. The organisms' growths were sub-cultured in nutrient agar to create pure cultures of different strains. The analysis was conducted on individuals with phlegm expectorating cough who had not taken any cough medications over a month. The research was not conducted on those who declined to give their free-willed consent.

*Identification and characterisation of bacterial isolates*

Actively growing cells were cultured in nutrient broth for 24 hours at 37 °C. A 1 mL portion of the samples was used for the antimicrobial test. The cells were suspended and diluted in nutrient broth to produce initial cell counts. After 24 hours, the appearance, shape, gram reactivity, and colour of each bacterial colony were examined using morphological and biochemical characterisation as reported by Fawole and Oso. [23]

*Indole test*

Escherichia coli was identified based on its ability to break down the amino acid tryptophan by releasing Indole. 0.5 mL of Kovac's reagent was added, and the mixture was gently shaken, showing a crimson colour on the upper layer.

*Hydrogen sulphide and catalase tests*

The method was used to identify *Proteus* species. For catalase, 3 mL of hydrogen peroxide solution was introduced into a test tube after the test organism was added to the mixture. Bacteria that test positive for hydrogen sulphide can synthesise hydrogen sulphide using inorganic sulphur compounds or sulphur-containing amino acids.

*Citrate test*

This test was carried out to differentiate between *Streptococcus spp*. and *Klebsiella spp. S*ome bacteria isolates get their carbon supply from citrate. The test organism was streaked on a slant and stabbed in the butt while it was floating in saline solution. After germination at 35°C for 48 hours, a blue colour emerged.

*Oxidase and Coagulase tests*

The coagulase test quantifies a bacteria isolate's inability to clot horse serum. The clot is found in the plasma by converting fibrinogen to fibrin. The oxidase test dictates the presence of a cytochrome oxidase system that facilitates the transfer of electrons between electron donors in bacteria.

*Antimicrobial activity determination*

The bacterial isolates were properly streaked on various plates after fully solidifying the media. The plate containing Gentamicin was used as the control. Both control and samples were labelled on each plate. The plant extracts were then streaked in different concentrations of 50 mg, 100 mg, and 200 mg on plates. The plates were kept overnight at 37 °C. After incubation, plates were examined for clear zones of

inhibition formed around them, signifying antimicrobial activity. Finally, measurements were taken to quantify the plate inhibition zones in millimetres.

*Total dehydrogenase activity assay*

The procedure for dehydrogenase assay was done as reported by Alisi *et al*.[6] Briefly, dehydrogenase activities result in the reduction of an artificial electron acceptor known as 2, 3, 5-triphenyl tetrazolium chloride (TTC) to red triphenyl formazan. (TPF). The microbial solutions and optical densities were standardised to 0.70 at 420 nm using a spectrophotometer and utilised as the inoculum for the dehydrogenase assay. Three glass containers were supplemented with 2.5 mL of glucose-broth medium in phosphate buffer with a pH of 6.8. Bacterial suspensions and plant extracts were inoculated at 50, 100, and 200 g/mL at 0.3 milliliters volume. The pre-incubation phase was conducted for 30 minutes in a rotary incubator rotating at 150 revolutions per minute. Subsequently, 0.1 mL of 1% (w/v) TTC solution in deionised water was administered to each test container. The mixture was subjected to an additional incubation period of 8.0 hours at room temperature, maintained at 28 ± 20°C. The maximum wavelength of TPF was obtained by isolating it in 4 mL of butanol and measuring its quantity using spectrophotometry at 500 nm. The output of formazan was calculated using a standard dose-response curve (0.20 g/mL TPF in butanol) with a linear equation of y = 0.175x and a high coefficient of determination (where R2 = 0.997). Dehydrogenase activity was quantified by measuring the amount of triphenyl formazan (TPF) produced per milligram of dry weight of cellular biomass within one hour. The comparative efficacy of plant extracts in inhibiting dehydrogenase activity was evaluated against the reference drug Gentamicin, calculated from the equation below.

$$\%\ Inhibition\ of\ DHA\ activity = 100 - \frac{\text{Absobance of test}}{Absorbance\ of\ Control}\ x\ 100$$

or = 100 – Percent DHA of control.
Where DHA = Dehydrogenase

## Statistics Analysis

Sigma Statistical System Software version 10 was used to analyse the percentage inhibitions of the plant extracts against various isolates. Results were displayed to determine the median inhibitory concentrations ($IC_{50}$) for all isolated microorganisms.

## Results and Discussion

The dispersion result of the organisms found in cough sputum is presented in Table 1. 13 out of the 22 clinical sputum samples cultured for examination exhibited growth on the plates. Three (3) sputum cultures showed no growth, while six (6) showed mixed growth due to multiple organisms in the plate.

**Table 1:** Distribution of organisms isolated from cough sputum samples

| Sample | Number of Organisms Isolated (CFU) | Percentage isolated (%) |
|---|---|---|
| Number showing growth on plates | 13 | 59.09 |
| Number without growth | 3 | 13.64 |
| Number with mixed growth | 6 | 27.27 |
| Total samples | 22 | 100 |

**Table 2:** Prevalence (%) of bacterial isolates yielding growth from cough sputum samples

| Isolates | Number isolated (CFU) | Prevalence (%) |
|---|---|---|
| Klebsiella species | 3 | 23.08 |
| Escherichia coli species | 2 | 15.38 |
| Streptococcus species | 6 | 46.16 |
| Proteus species | 2 | 15.38 |

**Figure 1**. *Gongronema latifoluim* leaves


**Figure 2**.*Piper guineense* leaves

**Figure 3.** *Vernonia amygdalina* leaves

**Figure 4**. Antibacterial activity on aqueous extract of *P. guineense* leaf against *Streptococcus pneumonia, Klebsiella pneumonia,* and *Proteus mirabilis and* Antibacterial activity on aqueous extract of *G. latifoluim* leaf against *Streptococcus spp, E.coli,* and *Klebsiella spp*

**Figure 5**. Antibacterial activity of aqueous extract of *V. amygdalina* leaves against cough sputum bacteria *Escherichia Coli, Klebiella spp,* and *Streptococcus spp.*

**Table 3:** Colonial Morphology and Biochemical Characteristics of cough sputum bacterial isolates.

| Colonial Morphology | Gram stain | Cell shape | Catalase test | Coagulase test | Oxidase test | Indole test | Citrate Test | Hydrogen sulphide test | Probable organism present |
|---|---|---|---|---|---|---|---|---|---|
| **Creamy-coloured, mucoid colonies** | + | Cocci in chains | _ | _ | _ | _ | + | _ | *Streptococcus species* |
| **Large circular creamy colonies** | _ | Rod | + | NA | _ | + | _ | _ | *Escherichia coli* |
| **Large circular creamy mucoid colonies** | _ | Rod | + | NA | _ | _ | + | _ | *Klebsiella species* |
| **Small, irregularly shaped mucoid colonies with green pigmentation** | _ | Rod | + | NA | _ | _ | _ | + | *Proteus species* |

**Table 4:** Zone of Inhibition (mm) produced by aqueous extracts of selected traditional anti-cough plants against cough sputum bacterial isolates

| ORGANISM | | PLANT EXTRACTS | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Piper guineense* | | | *Gongronema latifolium* | | | *Vernonia amygdalina* | | | Combined extract | | | Control (Gentamicin) |
| | Extract conc(mg/ml) | 200 | 100 | 50 | 200 | 100 | 50 | 200 | 100 | 50 | 200 | 100 | 50 | |
| | ISOLATES | | | | | | | | | | | | | |
| ***Streptococcus species*** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 28 |
| | 2 | 14 | 10 | 7 | 0 | 0 | 0 | 13 | 9 | 0 | 14 | 11 | 8 | 33 |
| | 3 | 11 | 8 | 0 | 14 | 11 | 8 | 11 | 7 | 0 | 14 | 9 | 0 | 24 |
| | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31 |
| | 5 | 13 | 8 | 7 | 0 | 0 | 0 | 12 | 10 | 7 | 12 | 10 | 8 | 26 |
| | 6 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 28 |
| ***Escherichia coli*** | 7 | 0 | 0 | 0 | 11 | 8 | 7 | 15 | 12 | 8 | 13 | 9 | 7 | 21 |
| | 8 | 0 | 0 | 0 | 12 | 0 | 0 | 13 | 10 | 8 | 12 | 8 | 0 | 36 |
| ***Proteus species*** | 9 | 13 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 |
| | 10 | 11 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31 |
| ***Klebsiella species*** | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 28 |
| | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 26 |
| | 13 | 12 | 0 | 0 | 0 | 0 | 0 | 15 | 11 | 9 | 13 | 9 | 7 | 34 |

**Keys:**
ISOLATES 1-6: Different Isolates of *Streptococcus* species
ISOLATES 7-8: Different Isolates of *Escherichia coli*
ISOLATES 9-10: Different Isolates of *Proteus* species
ISOLATES 11-13: Different Isolates of *Klebsiella* species

**Table 5:** Median threshold inhibitory concentration ($IC_{50}$) of *P. guineense, G. latifolium, V. amygdalina* extracts and ternary combination of extracts to dehydrogenase activity of *K. pneumonia, E. coli, S. pneumonia,* and *P. mirabilis* isolated from sputum samples

| Isolates | Plant Extracts | | | |
|---|---|---|---|---|
| | *Piper guineense* | *Gongronema latifolium* | *Vernonia amygdalina* | Combined extract |
| *Streptococcus spp.* | 39.09 | 23.69 | 19.53 | 19.53 |
| *Escherichia coli spp.* | 32.05 | 10.49 | 8.69 | 6.37 |
| *Proteus spp.* | 30.08 | 17.83 | 9.04 | 9.87 |
| *Klebsiella spp.* | 113.37 | 886.08 | 28.74 | 28.26 |

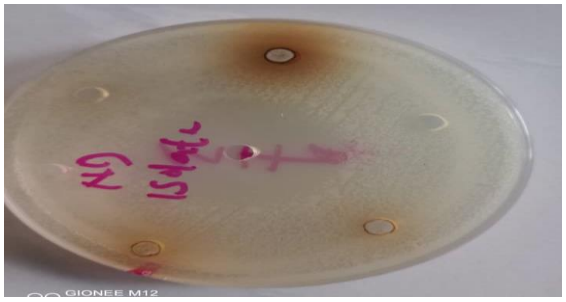

**Figure 6**. Antibacterial activity of aqueous extract of combined leaf extracts (PG+VA+GL) against cough sputum bacteria, *Streptococcus spp*, and *Klebsiella spp, E.coli*.

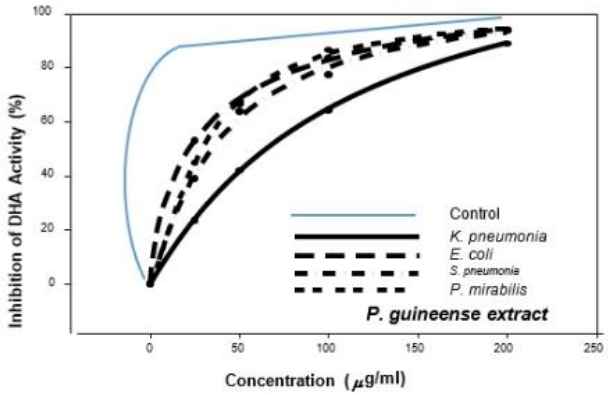


**Figure 7.** Dose-response plots of inhibitory effect of *P. guineense* extract to total dehydrogenase activity of *Klebsiella spp, E. coli, Streptococcus spp,* and *Proteus spp*. isolated from sputum samples.

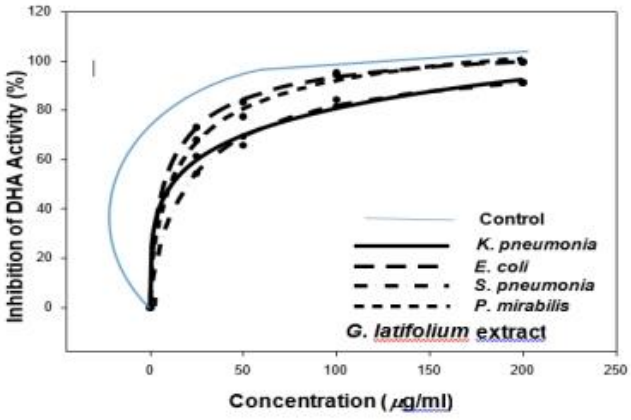


**Figure 8.** Dose-response plots of inhibitory effect of *G. latifolium* extract to total dehydrogenase activity of *Klebsiella spp, E. coli spp, Streptococcus spp,* and *Proteus spp.* isolated from sputum samples.

Table 2 presents the percentage and relative frequency of the bacterial strains and species found in the sputum samples. The percentage of *Streptococcus* species obtained was 46.16 %, *Klebsiella* species 19.6%, while *Escherichia coli* and *Proteus* species were 15.38% each. Table 3 lists the morphological and biochemical characterisation of the isolated bacterial species. The table demonstrates the presence of gram-positive, flat mucoid colonies, cocci in chains, and creamy-coloured *Streptococcus spp*. Three additional species were detected, all of which were gram-negative, rod-shaped, and catalase-positive. *Escherichia coli* was recognised as the bacterial that produced a positive Indole test result and large circular flat creamy colonies, whereas *Klebsiella spp*. produced a positive citrate test result and large circular creamy mucoid colonies. The final gram-negative organism, *proteus* spp, produced a positive hydrogen sulphide ($H_2S$) test result and manifested as tiny, irregularly shaped mucoid clusters with green pigmentation. The results presented in Table 4 illustrate the various zones of inhibition, measured in millimetres, observed in the test organisms upon exposure to varying concentrations of 50, 100, and 200 mg/mL. The table indicates that out of the total 13 bacterial isolates, *Streptococcus spp., E. coli, Proteus spp, and Klebsiella spp* were isolated. The isolates were most sensitive to the combined extract and *V. amygdalina*. *Klebsiella and Proteus* species demonstrated the lowest susceptibility to this extract, while *Streptococcus* exhibited a higher susceptibility. The median inhibitory concentrations ($IC_{50}$) of the extracts are shown in Table 5. Compared to *P. guineense* and *G. latifolium*, the combined extract and *V. amygdalina* demonstrated greater inhibitory effects at lower concentrations as seen in $IC_{50}$ values of 88.08 and 113.37, *G. latifolium* and *P. guineense* were the least susceptible to *Klebsiella spp.*

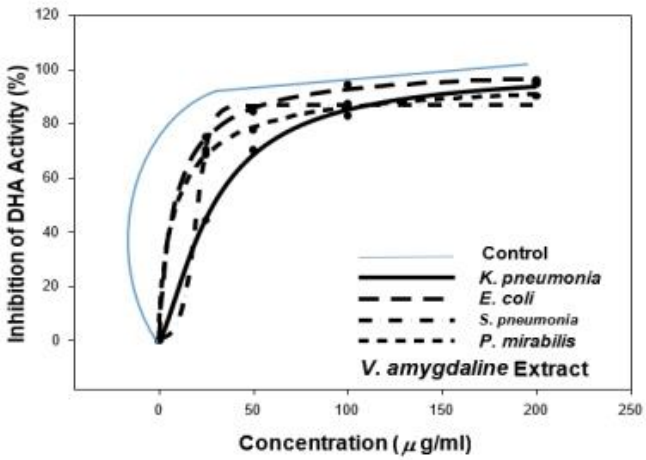


**Figure 9**. Dose-response plots of inhibitory effect of *V. amygdalina* extract to total dehydrogenase activity of *Klebsiella spp, E. coli spp, Streptococcus spp,* and *Proteus spp.* isolated from sputum samples.

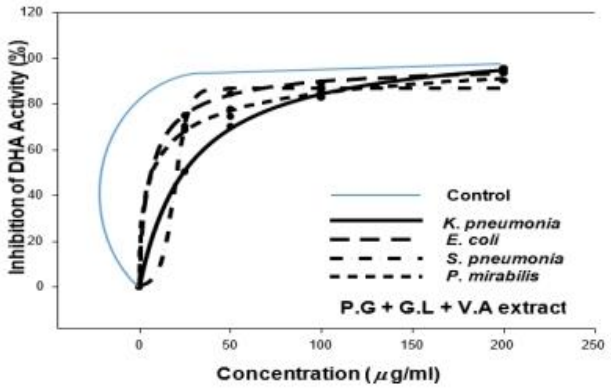


**Figure 10.** Dose-response plots of inhibitory effect of a ternary mixture of *P. guineense, G. latifolium,* and *V. amygdalina* extract to total dehydrogenase activity of *Klebsiella spp, E. coli, Streptococcus spp,* and *Proteus spp.* isolated from sputum samples.

The results in Figures 7 to 10 demonstrate the effect of single and combined aqueous extracts of anti-cough plants on reducing total dehydrogenase activity against *E. coli spp., Streptococcus spp., Klebsiella spp.,* and *Proteus spp*. The extracts inhibited the total dehydrogenase activities of the isolated organisms in a dose-dependent manner, adhering to the logistic dose-response curve. However, the extracts caused a lower extent of inhibition than the control drug, Gentamicin. The maximum inhibition of total dehydrogenase activity was observed at the highest extract concentrations. Tables 1 and 2 illustrate the distribution pattern of microorganisms in clinical sputum cough samples. The present study identified *Streptococcus and Klebsiella* as the most prevalent microorganisms. The results aligned

with a previous study that identified *Streptococcus pneumoniae* and *Klebsiella pneumoniae* as the most frequently encountered pathogens in cough patients.[24, 25] The bacterial isolates were identified as *E. coli*, *Klebsiella*, *Proteus*, and *Streptococcus species* based on their colonial morphology and biochemical characteristics. The colonies of these isolates exhibited variations in their morphological characteristics, including their shape, size, texture, and pigmentation. The identified gram-negative bacteria were *Escherichia coli*, *Klebsiella spp*, and *Proteus spp*, whereas *Streptococcus spp w*as the only gram-positive bacterium detected. These microorganisms have been frequently detected in clinical sputum samples and recognised as the primary causative agent of cough.[27,2]

The bacterial isolates responded to the plants' single and combined aqueous extracts in various concentrations. The plant extracts demonstrated inhibitory effects against *Streptococcus spp* (isolates 2, 3, and 5) at 50, 100, and 200 mg/ml concentrations. This aligns with the assertion that gram-positive bacteria exhibit greater susceptibility to plant-derived compounds and antibiotics than gram-negative bacteria. [27] The susceptibility of *E. coli* (isolates 7 and 8) was significant as observed in dose-dependent inhibition zones against the combined extract and *V. amygdalina*. Only the highest dosage of *P. guneense* inhibited the *Proteus spp* isolates, while *P. guneense*, *V. amygdalina*, and the combined extract inhibited the *Klebsiella spp* isolates. Hence, the aqueous leaf extract of *V. amygalina* and combined extracts exhibited the most effective inhibitory effects against the isolates, buttressing that *Vernonia amygdalina* possesses antibacterial properties. [27, 28,29] This study's findings provide additional evidence supporting the assertions regarding the antimicrobial properties of *V. amygdalina* extracts. The isolated organisms' dehydrogenase enzyme changed triphenyl tetrazolium chloride to triphenyl formazan, which helped assess the plant extracts antimicrobial properties. Given their low $IC_{50}$, *Proteus spp* and *E. coli spp* demonstrated the highest inhibition rates. While *Klebsiella spp* demonstrated the highest levels of resistance to the plant extracts as evidenced by its high $IC_{50}$ concentrations, *Streptococcus spp* exhibited moderate inhibition rates. This is consistent with the findings, which revealed that gram-negative bacteria exhibited greater dehydrogenase activity than gram-positive bacteria. [30, 31] The inhibitory effects of the combined extracts and *V. amygdalina* were higher than those of *P. guineense and G. latifolium.* Phytochemicals and antioxidants such as saponins, alkaloids, cardiac glycosides, and tannins may be responsible for the observed inhibitory effect in plants.

## Conclusion

According to the research, *Streptococcus spp*, *E. coli*, *Proteus spp*, and *Klebsiella spp* were found in clinical sputum samples. The effects of the plant extracts on the bacterial isolates typically range from *V. amygdalina* > *combined extract* > *P. guineense* > *G. latifolium*. *Streptococcus spp* and *Klebsiella spp* were the bacteria strains most susceptible to the plant extracts. The effects of various extracts on the dehydrogenase activity of the bacterial strains, as seen in the increasing sigmoid curve, indicate that the extracts impacted isolated organisms at increasing dose-dependent concentrations. Hence, the research explains the use of plants as cough suppressants and demonstrates that they have bactericidal effects.

## Conflict of interest

The authors declare no conflict of interest

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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