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Harnessing The Nature's Arsenal: Investigating The Antibacterial And Antifungal Properties Of Black Pepper Vines

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Abstract: The present work has been undertaken with the aim to determine the antifungal and antibacterial activity of dried extract obtained from the plant *Piper nigrum.* Two extracts of the *Piper nigrum* were prepared by Soxhlet extraction using ethanol and chloroform. Phytochemical screening of plant extracts showed the presence of alkaloids, glycosides, and carbohydrates. The work has also emphasized determining the antifungal activity of each plant extract against *Candida albicans* (fungi). Zone of inhibition of plant extracts with respect to three concentrations viz. 0.25 µg/ml, 0.5 µg/ml, and 1.0 µg/ml have been done and the zone of inhibition were observed. Comparing the above three concentrations for anti-fungal activity 0.5µg/ml shows maximum activity for ethanolic extract of *Piper nigrum* and 0.25 µg/ml shows maximum activity for chloroform extract of *Piper nigrum*. Zone of inhibition of plant extracts with respect to three concentrations viz. 0.25µ g/ml, 0.5µg/ml, 1.0µg/ml were performed and the zone of inhibition was observed. Comparing the above three concentrations for anti-bacterial activity 0.5µg/ml shows maximum activity for ethanolic extract of *Piper nigrum* and µg/ml shows maximum activity for chloroform extract of *Piper nigrum.* The result obtained were promising for the management of bacterial and fungal infections.

I. INTRODUCTION

Piper nigrum also known as Indian Black Pepper, is a flowering vine belonging to the family Piperaceae that is used for its fruit. It has a hot, bitter and sharp taste. The fruits are dried and used as a spice and flavor. It is also used as digestive, stomachic, emmenagogue, abortifacient, liver tonic, aphrodisiac, etc. In addition to fruits, the roots and thicker parts of the stem are also used in Ayurvedic and Unani medicines. The main active constituent present in black pepper is piperine. Other active constituents of black pepper include myrcene, eugenol, α- and β-pinene, α-phellandrene, limonene, linalool, methyl propenal, butyric acid, etc. The compounds present in the essential oil of black pepper fruit includes β-caryophyllene, limonene, sabinene, α- and β-pinene, caryophylleneoxide, terpinen-4-ol, 3-carene, copaene, elemene, α-copaene, naphthalene, etc.[1] With its aerial roots, this woody vine may reach up to 33 feet in the air. *Piper nigrum* grows best in areas with a lengthy wet season, moderate temperatures, and some shade. Its tiny blooms are packed closely together along thin stalks, with around fifty flowers per stalk. This results in drupes, which are simple, fleshy fruits with a single seed that are about a quarter of an inch in diameter. On a single stem, up to 30 fruiting spikes might be seen.[2]

Black pepper (*Piper nigrum*) is not only a popular spice known for its pungent flavor but also possesses significant antimicrobial properties. The antimicrobial activity of black pepper is primarily attributed to its bioactive compound called piperine. Piperine has been shown to exhibit antibacterial, antifungal, and even antiviral effects, making black pepper a versatile natural antimicrobial agent. Studies have demonstrated that piperine from black pepper can inhibit the growth of various bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Bacillus cereus*. These bacteria can cause foodborne illnesses and infections in humans. By inhibiting their growth, piperine helps to reduce the risk of microbial contamination in food and potentially contributes to food preservation.[3]

In addition to its antibacterial properties, black pepper and piperine also show antifungal activity against fungal strains like *Candida albicans*, which is responsible for yeast infections in humans. This broad-spectrum antimicrobial activity highlights the potential of black pepper as a natural alternative to synthetic antimicrobial agents. Furthermore, black pepper has been investigated for its synergistic effects with other antimicrobial compounds. Combining piperine with antibiotics has shown enhanced antimicrobial activity against certain drug-resistant bacteria, suggesting a possible role in combating antibiotic resistance.

Overall, black pepper is not only a culinary delight but also a potent antimicrobial agent due to its active component, piperine. Its ability to inhibit the growth of bacteria and fungi underscores its potential applications in food preservation, healthcare, and potentially in developing new antimicrobial strategies against infectious diseases.

II. MATERIALS AND METHODS

1. Procurement of the Plant material:

Piper nigrum were collected from the backyards of the households of Wayanad region and the herbarium was prepared. Then it was authenticated by botanist Dr. Raji. R, Prof. St.Mary's College of Arts and Science, Sulthan bathery. Vines and leaves of *Piper nigrum* were collected and then cut with sharp blades, pressed and dried in shade for preparing the herbarium.

Dried plant extract is diluted in chloroform and ethanol to get concentrated extract. It is then diluted with DMSO. Make dilutions with concentrations 0.25µ g/ml, 0.5µg/ml, 1.0µg/ml for antibacterial activity and 0.25µ g/ml, 0.5µg/ml, 1.0µg/ml for antifungal property.

2. Phytochemical screening:[4]

a) **Test for Alkaloids:**

Dragendorff's test – To the DMSO extract of the plant sample add a few drops of the Dragendorff's reagent and yield an orange to orange red precipitate if the alkaloids are present.
Mayer's test – To the DMSO plant extract add a few drops of Mayer's reagent and if it yields yellowish white precipitate that is an indication of Alkaloids.
Hager's test – To the DMSO plant extract add a few drops of Hager's reagent and if it yields yellow precipitate that is an indication of Alkaloids.
Wagner's test -to the 200 microliters of the latex in the test tube add a few drops of Wagner's reagent to the sides of the test tube to yield a reddish-brown precipitate indicate the presence of Alkaloids.

b) **Test for Carbohydrates:**

Molisch's test – To the DMSO extract of the latex, add 2-3 drops of Molisch's reagent slowly and concentrated sulphuric acid along the sides of the test tube, formation of a violet ring confirms the presence of carbohydrate.
Fehling's test – to the test tube add 1 ml sample and 1 ml mixture of fehling's A and B solution and heat the test tube. Development of a red precipitate indicates the prescence of carbohydrate.
Benedict's test- To the test tube add 1 ml of the sample and 1 ml benedict's solution and heat the test tube development of brick red precipitate indicates the prescence of carbohydrate.
Barford's test – to the sample add barford's reagent and theformation of red colourat the base of the test tube indicates the prescence of carbohydrate.

c) **Test for phenolic compounds:** ferric chloride test: to DMSO extract of the latex, add a few drops of 5% ferric chloride along the sides of the test tube, a dark green color showed due to the presence of phenolic compounds.

3. Antifungal study: Agar well diffusion method[5]

20 ml of sterile culture media were poured into Petri plates. Then 1ml of inoculum suspension of *C. albicans* was spread over the medium. Make a well of 6mm by using a sterile cork borer. Then add 100 microlitres of extract. Incubate the Petri plates for 48 hours at 37C. Measure the zone of inhibition of each Petri plate. The standard used in this study is Flucanazole (0.25mg/L as MIC).

4. Antibacterial study: Agar well difusion method)[6]

20 ml of sterile culture media were poured into Petri plates. Then Iml of inoculum suspension of E. coli was spread over the medium. Make a well of 6mm by using a sterile cork borer. Then add 100 microlitres of extract. Incubate the Petri plates for 24 hours at 37°C. Measure the zone of inhibition of each Petri plate. The standard used in this study is Ampicillin (4mg/L as MIC).

Standard drug:

- CHLORAMPHENICOL - Antibacterial standard
- FLUCANAZOLE- Antifungal standard

Solvent:

- DMSO

Microorganism:

- *Candida albicans*
- *Escherichia coli*

III. RESULTS

. **1. Procurement of the Plant material**:

Piper nigrum were collected from the backyards of the households of Wayanad region and the herbarium was prepared. Then it was authenticated by botanist Dr. Raji. R, Prof. St.Mary's College of Arts and Science, Sulthan bathery. Vines and leaves of *Piper nigrum* were collected and then cut with sharp blades, pressed and dried in shade for preparing the herbarium.

Dried plant extract is diluted in chloroform and ethanol to get concentrated extract. It is then diluted with DMSO. Make dilutions with concentrations 0.25µ g/ml, 0.5µg/ml, 1.0µg/ml for antibacterial activity and 0.25µ g/ml, 0.5µg/ml, 1.0µg/ml for antifungal property.

2. QUALITATIVE PHYTOCHEMICAL SCREENING

The qualitative chemical tests for *Piper nigrum* were performed and the results are as follows:

Sr. No.	TESTS	RESULTS
1	Test for Alkaloids	+++
2	Test for Carbohydrates	+++
3	Test for Phenolic compounds	++
4	Test for Saponins	-

The plant extracts obtained from *Piper nigrum* was subjected to preliminary phytochemical analysis, revealing the presence of various chemical constituents such as Alkaloids, Carbohydrates, Phenolic compounds, etc. The phytochemical screening confirms the presence of Anti-fungal components, with alkaloids potentially contributing to antimicrobial activity

The Qualitative chemical screening tests for *Piper nigrum*.

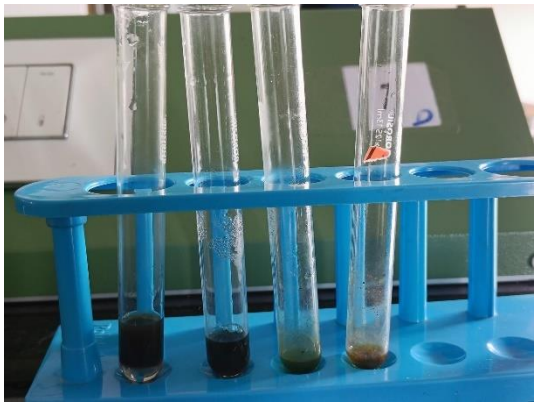


Figure 2.1. Test for Alkaloids using the extract of *Piper nigrum* dried vines

Figure 2.2. Test for Carbohydrates using the extract of *Piper nigrum* dried vines

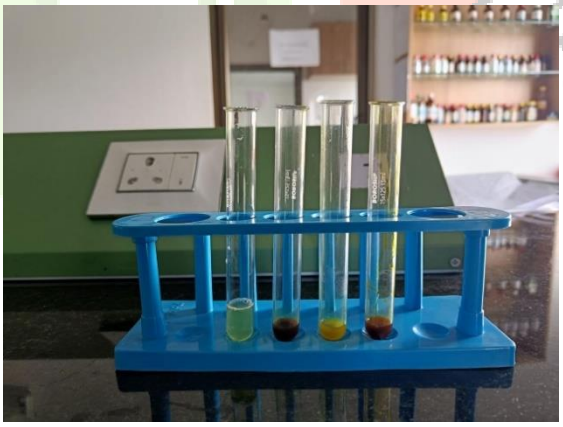


Figure 2.3. Test for phenolic compounds using the extract of *Piper nigrum* dried vines

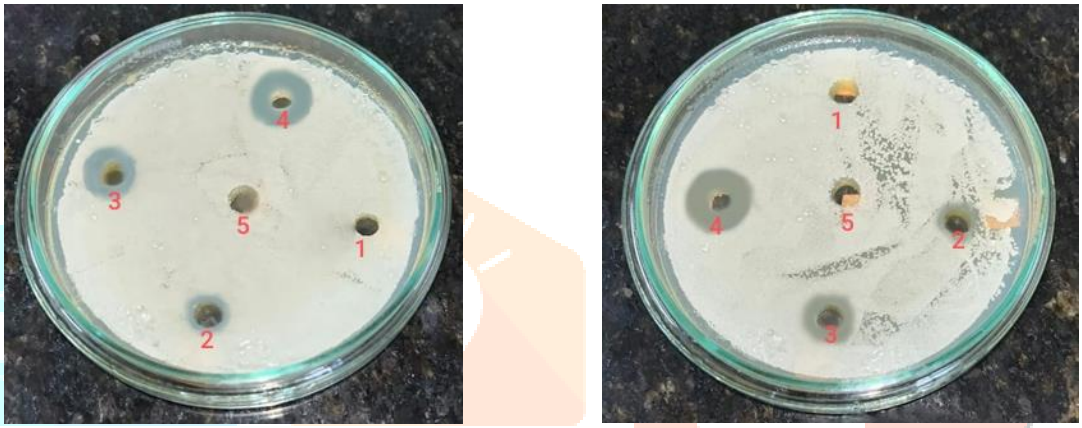
3. ANTIFUNGAL ACTIVITY: (*Candida albicans*)

Anti-fungal activity of dried vine extract obtained from *Piper nigrum* has been carried out.

Table 3.1 Zone of inhibition (diameter) of C. albicans by Piper nigrum

DILUTION (mg/L)	ZONE OF INHIBITION	
	ETHANOL	CHLOROFORM
0.25	5 mm	6 mm
0.5	10 mm	11 mm
1	14 mm	13 mm
DMSO	-	-
standard (Fluconazole)	22 mm	22 mm

p value < 0.05, thus it is significant



(A) **(B)**

Figure 3.1. Zone of inhibition of *Candida albicans* by different concentrations of *Piper nigrum* in the Ethanolic extract (A) and Chloroform extract (B) respectively.
1: 0.25 µg/ml; 2: 0.50 µg/ml; 3: 1.0 µg/ml; 4: Standard; 5: DMSO

Zone of inhibition of the Ethanolic extract of *Piper nigrum* of three concentrations such as, 0.25µ g/ml, 0.5µg/ml and 1.0µg/ml has been done and the diameters observed are 5 mm, 10 mm and 14 mm respectively.

Zone of inhibition of the Chloroform extract of *Piper nigrum* of three concentrations such as, 0.25µ g/ml, 0.5µg/ml and 1.0µg/ml has been done and the diameters observed are 6 mm, 11 mm and 13 mm respectively. The standard drug Fluconazole was taken in the concentration 1 mg/ml and the diameter was found to be 22 mm.

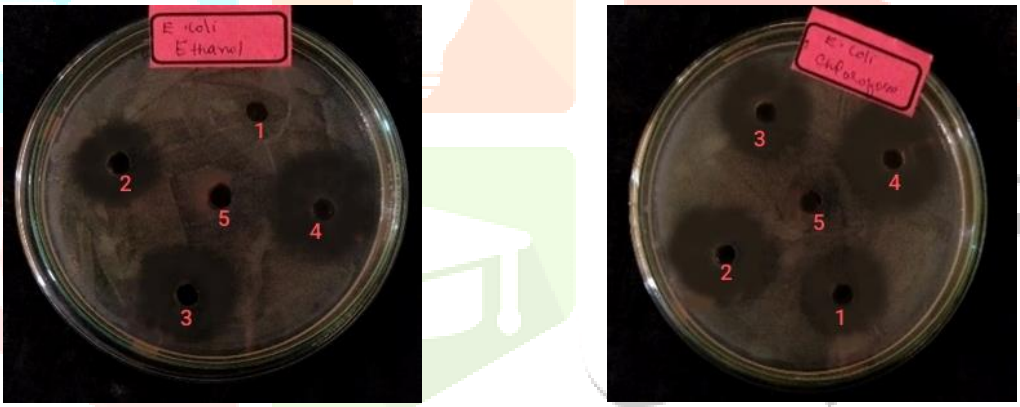
4. ANTI BACTERIAL ACTIVITY: (*Escherichia coli*)

Anti- bacterial activity of extract obtained from *Piper nigrum* has been carried out

Table 4.1 Zone of inhibition (diameter) of E. coli by Piper nigrum

DILUTION (mg/L)	ZONE OF INHIBITION [DIAMETER (mm)]	
	ETHANOL	CHLOROFORM
0.25	5 mm-	11 mm
0.5	12 mm	14 mm
1	16mm	16 mm
Undiluted extract	-	-
Standard (Chloramphenicol)	23 mm	23 mm

p value < 0.05, thus it is significant



(A) **(B)**

Figure 4.1. Zone of inhibition (diameter) of *E. coli* by different concentrations of *Piper nigrum* in the Ethanolic extract (A) and Chloroform extract (B) respectively.
1: 0.25 µg/ml; 2: 0.50 µg/ml; 3: 1.0 µg/ml; 4: Standard; 5: DMSO

Zone of inhibition of the Ethanolic extract of *Piper nigrum* of three concentrations such as, 0.25µ g/ml, 0.5µg/ml and 1.0µg/ml has been done and the diameters observed are 5 mm, 12 mm and 16 mm respectively.

Zone of inhibition of the Chloroform extract of *Piper nigrum* of three concentrations such as, 0.25µ g/ml, 0.5µg/ml and 1.0µg/ml has been done and the diameters observed are 11 mm, 14 mm and 16 mm respectively. The standard drug Chloramphenicol was taken in the concentration 1 mg/ml and the diameter was found to be 23 mm.

5. ANTI BACTERIAL ACTIVITY: (*Staphylococcus aureus*)

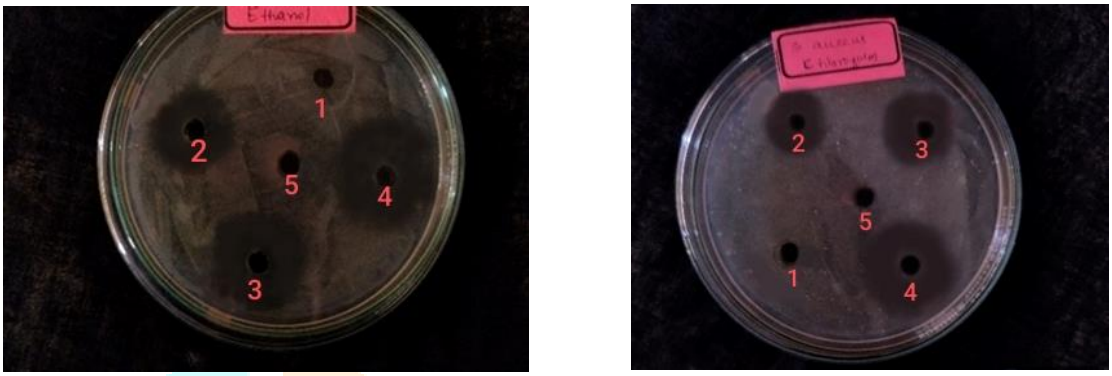
Anti- bacterial activity of extract obtained from *Piper nigrum* has been carried out

Table 5.1 Zone of inhibition (diameter) of S. aureus by Piper nigrum

DILUTION (mg/L)	ZONE OF INHIBITION [DIAMETER (mm)]	
	ETHANOL	CHLOROFORM
0.25	12 mm	No

0.5	14 mm	15 mm
1	17 mm	10 mm
Undiluted extract	-	-
Standard (Chloramphenicol)	24 mm	24 mm

p value < 0.05, thus it is significant



(A) **(B)**

Figure 5.1. Zone of inhibition (diameter) of *S. aureus* by different concentrations of *Piper nigrum* in the Ethanolic extract (A) and Chloroform extract (B) respectively.
1: 0.25 µg/ml; 2: 0.50 µg/ml; 3: 1.0 µg/ml; 4: Standard; 5: DMSO

Zone of inhibition of the Ethanolic extract of *Piper nigrum* of three concentrations such as, 0.25µ g/ml, 0.5µg/ml and 1.0µg/ml has been done and the diameters observed are 12 mm, 14 mm and 17 mm respectively.

Zone of inhibition of the Chloroform extract of *Piper nigrum* of three concentrations such as, 0.5µg/ml, 1.0µg/ml has been done and the diameters observed are 15 mm and 10 mm, respectively while no zone of inhibition was observed for 0.25µ g/ml. The standard drug Chloramphenicol was taken in the concentration 1 mg/ml and the diameter was found to be 24 mm.

DISCUSSION

Candida albicans, a prevalent opportunistic fungal pathogen, plays a crucial role in causing various infections, particularly in immunocompromised individuals. Its affinity to switch between different morphological forms and its arsenal of virulence factors make it a formidable adversary in the clinical settings. The infection caused by Candida albicans, can manifest in multiple forms, ranging from superficial mucosal infections to systemic diseases, Moreover, the rise in antifungal resistance poses a serious challenge in the management of these infections. Therefore, understanding the biology and pathogenicity of Candida albicans, along with the mechanisms of action and limitations of current antifungal therapies, is essential to develop effective strategies to combat this medically significant fungal pathogens.[7] Fluconazole is effective against the majority of the Candida species, including Candida albicans, which was chosen as the control in the study and showed a MIC Value of 0.25 mg/L.

Escherichia coli (E. coli) is a Gram-negative bacterium commonly found in the intestines of humans and animals. While it plays a vital role in various biological processes, certain strains of E. coli can cause a range of infections, including urinary tract infections, gastroenteritis, and bloodstream infections.[8] The clinical management of E. coli infections primarily relies on antibiotic therapies. However, the emergence and spread of antibiotic resistance have become significant challenges in treating E. coli infections effectively. Understanding the mechanisms of antibiotic resistance and exploring alternative strategies, such as new drug development and combination therapies, are crucial for combating this pathogen and improving patient outcomes.[9] Chloramphenicol is widely used to treat human and livestock E. coli infection and it is utilized as the control in the study with MIC value of 4mg/L.

Different phytochemicals with biological activity can be found in a variety of herbs and herbal extracts, offering therapeutic value. The therapeutic effects of plants are attributed to these phytochemicals, which are plant compounds that do not provide nutrition. For instance, saponins, terpenoids, flavonoids, tannins, and

alkaloids have anti-inflammatory effects, while glycosides, flavonoids, tannins, and alkaloids possess hypoglycemic effects. Steroids and triterpenoids display analgesic effects, and steroids and saponins are responsible for central nervous system activity. Conducting a phytochemical survey is beneficial for assessing the active biological components of Medicinal plants.

The extract obtained from the dried vines of subjected to preliminary phytochemical analysis, revealing the presence of chemical constituents such as alkaloids, phenolic compounds, carbohydrates, saponins, etc. which confirms the presence of anti-fungal components (phenolic compounds), with alkaloids potentially contributing to antimicrobial activity.

The agar well diffusion assay is a standard method widely used for the rapid screening of natural products for antimicrobial activity. Plant latexes were screened using this very convenient assay method. The results indicate that caution is needed, since the extracts may have different diffusion rates on the agar plate, and this may contribute to variations in the size of the inhibitory zones, leading to erroneous conclusions regarding their antifungal activity. *Piper nigrum* shows its antifungal activity at concentrations of 0.25µg/ml, 0.50 µg/ml and 1 µg/ml with the zone of inhibition of 5 mm, 10mm and 14 mm respectively. Fungal activity is considered as a desirable quality for antifungal agents since it can eliminate the fungus from tissues.

The chloroform extract of *Piper nigrum* shows its antibacterial activity at concentrations of 0.50µg/ml, 1µg/ml with zone of inhibition of 15 mm and 10mm respectively and no zone of inhibition was obtained at 0.25 µg/ml.
The ethanolic extract of *Piper nigrum* shows its antibacterial activity at concentrations of 0.25 µg/ml, 0.50 µg/ml and 1µg/ml with zone of inhibition of 12 mm, 14 mm, and 17 mm respectively.

In the comparative study of both the extracts, it was found that ethanolic extract of *Piper nigrum* shows its antifungal activity at a concentration of 0.5 µg/ml with a zone of inhibition of 15 mm, while chloroform extract shows its antifungal activity at a concentration of 1 µg/ml with a zone of inhibition of 15mm.

And for antibacterial comparative study, Ethanolic extract of *Piper nigrum* shows its antibacterial activity at a concentration of 8µg/ml with a zone of inhibition of 30mm, while chloroform extract shows its antibacterial activity at a concentration of 2ug/ml with a zone of inhibition of 20mm.

We studied the efficacy of two extracts for their activity against *Candida albicans* by In-vitro method. The chloroform extract of *Piper nigrum* was found to be more effective for its antifungal activity than Ethanolic extract, while Ethanolic extract of *Piper nigrum* was found to be more effective for its antibacterial activity than the chloroform extract.

Fluconazole are known to be very effective against human pathogenic fungi and bacteria and despite their severe side effects, may require prolonged use. It is encouraging to note that both the extracts obtained from *Piper nigrum* was found to be fungicidal at low concentrations. Until now not much information was available about the mode of action of natural products that inhibit Candida growth. Plant extract could find use as an anti-candida agent against azole-resistant strains. Most of the plant latexes have a long history of use in food, confectionery, and as a component of perfume. However, before they are considered for use as a topical preparation, a careful exploration of their undesirable effects needs to be undertaken.

IV. SUMMARY AND CONCLUSION

In summary, the result presented in this paper demonstrates the antifungal potential of selected extracts of *Piper nigrum*. The ethanolic extracts was found to be more effective for its antifungal activity than chloroform extract, while Chloroform extract was found to be more effective for its antibacterial activity than ethanolic extract. These results not only encourage further examination of the efficacy of plant extract against other forms of systemic and superficial fungal infections but also the exploration of their broad-spectrum effects against other pathogenic manifestations, including malignancies, in the long years.

In conclusion, the comparative study of ethanolic extract and chloroform extract of the dried vines of *Piper nigrum* for the management of bacterial and fungal infections has provided a valuable insight into potentials of such natural sources as additives in the antifungal and antibacterial formulations.

Through investigations, it was found that *Piper nigrum* contains several bioactive compounds, which demonstrated inhibitory effects in the growth of the bacterial and fungal cultures, which indicates their suitability for clinical applications.

Nonetheless, this comparative study serves as a foundation for future investigations contributing to the growth as a natural source in the development of novel antifungal and antibacterial agents.

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