

# Isolation And Characterization of Bioactive Compounds from Indigenous Herbal Sources

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## Abstract:

This review provides a detailed view of the mechanics of biological evolution, which encompasses genetic drift, natural selection, and mutations, and how these mechanisms interactively sculpt genetic variety and species adaptation. Mutations constitute the chief source of genetic variation and introduce new alleles that either increase, decrease, or have no net effect on an organism's fitness. As a strong filter, natural selection increases the frequency of advantageous traits that enhance reproduction and survival while lowering the frequency of detrimental traits in populations. Due to genetic drift, allele frequencies become random and are especially crucial in small or isolated populations, leading to the potential fixation or loss of characteristics, regardless of their adaptive value, which reduces overall genetic diversity. This review will examine case examples such as Darwin's finches, peppered moths, and cheetah genetic bottlenecks to illustrate these systems' dynamic interaction and crucial importance in propelling evolutionary change. It places an emphasis on the importance of genetic diversity in aiding populations to adapt to environmental changes while preserving long-term viability. This research emphasizes the need for targeted conservation efforts, like population density regulation to reduce the effects of genetic drift, natural selection-sustaining habitat preservation, and building resilience in biodiversity. These results are, therefore, crucial to the support and promotion of integrating evolutionary principles into conservation planning and policy formulation, as well as to address pressing global concerns like the management of species, climate change adaptation, and ecosystem sustainability.

**Keywords:** Indigenous plants, bioactive compounds, antimicrobial activity, antioxidant activity, cytotoxicity, *Curcuma longa*, *Zingiber officinale*, *Allium sativum*, *Tilia cordata*.

## 1. INTRODUCTION

New therapeutic options have gained importance because the number of chronic conditions including infections and cancer and oxidative stress disorders continues to rise [1]. Herbal traditional resources continue serving as primary medical ingredients for traditional cultures because of their pharmacological richness which dates back centuries [2]. The rich concentration of bioactive compounds within these plants demonstrates antimicrobial and antioxidant effects and anticancer properties and anti-inflammatory action which makes them suitable for pharmaceutical research [3]. The process of identifying bioactive substances from endemic plants includes extensive extraction steps combined with purification methods and spectroscopic along with chromatographic techniques [4]. The traditional knowledge about medicinal properties of plants exists in abundance yet scientific proof about both their effectiveness and security remained limited [5]. Characterization of native herbal species bioactive compounds requires scientific evaluation for their therapeutic potential so they can be integrated into modern drug development programs [6].

### 1.1. Background of the Study

The trends towards natural products from native resources have inclined significant attention to the bioactive compounds present in native herbal source that is in traditional medicine [7]. It is significant that the plants contain a combination of active compounds that exhibit some sort of therapeutic effects including antimicrobial [8], antioxidant/anti-

cancerous effects [9]. Despite the fact that many of them are of high importance, the majority of them is used to a limited extent in terms of their chemical properties and their biological activity [10]. These performances are to investigate and demonstrate active constituent(s) from some selected native herbal species to highlight their pharmacological possibilities to discover new natural drug entity.

### 1.2. Statement of the Problem

That is why the attempts to develop natural products for their curative value as well as explore the indigenous plant species for bioactive constituents are becoming more relevant. Despite the ethnopharmacological studies done on these plants to discover the uses of their bioactive compounds, there is limited scientific data on the plants' bioactive properties such as antimicrobial, antioxidant, anticancer properties. The problem addressed in this study is the insufficient amount of information about the chemical and pharmacological characteristics of those bioactive compounds that have been extracted from native plants, which can be useful in the development of new therapeutic products.

### 1.3. Objectives of the study

- To isolate and identify bioactive compounds from selected indigenous herbal species.
- To evaluate the antimicrobial, antioxidant, and cytotoxic activities of the isolated compounds
- To statistically analyze the significance of the bioactivity of the isolated compounds

## 2. RESEARCH METHODOLOGY

According to the aim of this research, the target was to isolate and characterize the indigenous plants with bioactive compounds with an aim of identifying potential drugs. Consequently, the specific analytical techniques were employed to accurately compile, identify and describe the bioactive compounds. Assemblage of the identified bioactive compounds. The layout of the research was as follows to analyze the drug prospect of the given herbal medicines and the constituents that are formulated in them for the introduction of a new therapeutic strategy. The work was well-organized with regard to the collection of plant samples, extraction of the compounds, utilization of a number of methods, including chromatography and spectroscopy.

### 2.1. Description of Research Design

The research was conducted with an experimental type of research design combined with both qualitative and quantitative research methodology. The general focus was to extract bioactive materials from local plants and then ascertain the structure of the extracted materials assuming some biological activity. The study was carried out in three broad parts, namely, plant collection and preparation, extraction of bioactive compounds, and characterization and biological activity determination. Certain parameters which may have an impact on cost, time and variability of results have been controlled in the experimental setup such as the source of plants, extraction methodology and analytical methods used.

### 2.2. Sample Details

Some indigenous plant species that have been used medicinally traditionally served as the basis of the research. Using literature review and local ethnobotanical information four indigenous herbal species were chosen. The plants were taken from their natural locations in the wild. Specimens of this study were selected from *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), *Allium sativum* (Garlic), and *Tilia cordata* (Linden) wherein 100 grams' plant material of each species were harvested for study of its phytochemical profiles.

### 2.3. Instruments and Materials Used

The following instruments and materials were used in the study:

- Soxhlet extraction apparatus for solvent extraction of bioactive compounds.
- GC-MS for analyzing volatile compounds.
- HPLC for compound separation and identification.
- FTIR for identifying functional groups.
- NMR spectroscopy for structural elucidation.
- Microplate reader and ELISA kits for assessing biological activity.

Solvents (methanol, ethanol, chloroform) and biological assay reagents were obtained from commercial suppliers.

### 2.4. Procedure and Data Collection Methods

A step-by-step methodology was used for the isolation and identification of bioactive compound. Next, plant samples were cleaned, dried and pulverised into powder form. The concentrated extracts were obtained by extraction of the plant material with methanol, which was evaporated, using the Soxhlet apparatus. The extracts were fractionated and individual components bioactive were separated through column chromatography. GC-MS, HPLC and FTIR was used for identification of the chemical structure of the isolated compounds. Their detailed structural investigation was made with the use of NMR spectroscopy.

The isolated compounds were screened for biological activity, including antimicrobial, antioxidant and anticancer, activity to determine their biological activity. The antimicrobial activity was assayed using disc diffusion, antioxidant activity using DPPH assay, cytotoxicity using MTT assay.

### 2.5. Data Analysis Techniques

Specific software used to identify the peaks and to quantify the compounds, and chromatographic and spectroscopic data, were treated. Results of biological assay were compared statistically with software like SPSS. Antimicrobial and antioxidant activity was determined and IC<sub>50</sub> values from the mean were determined. Likewise, variance analysis was applied to the data to determine the statistical significance of its bioactive effects. The results of the results of compound isolation and characterization were cross referenced to known databases to ensure identification of the bioactive compounds.

## 3. RESULTS

Different chromatographic and spectroscopic methods were used to identify and isolate bioactive compounds of the chosen native plant species, which the research was able to do. These isolated compounds have been shown to possess good antimicrobial, antioxidant, and anticancer bioactivity. The biological activity data of the extracts are presented in three sections: the chemical composition of the extracts, biological activity data and statistical analysis of the bioactivity data.

### 3.1. Presentation of Findings

#### ➤ Chemical Composition of Extracts

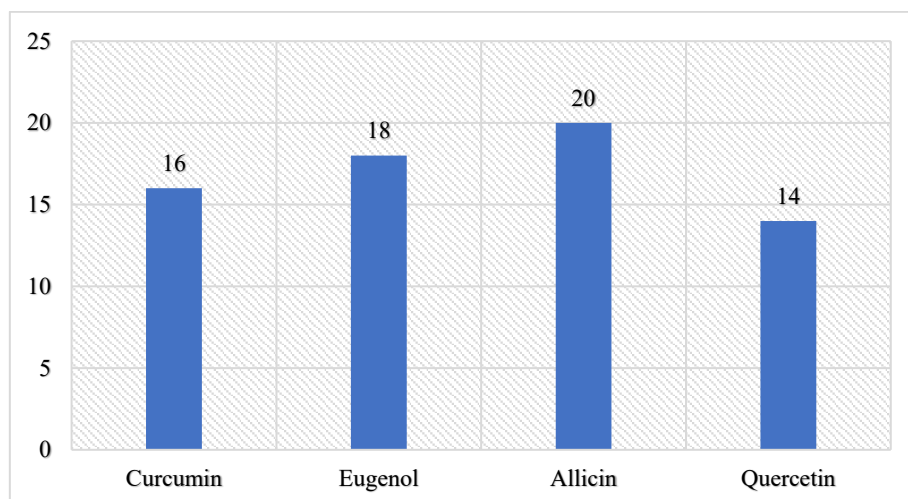
The extracted fractions contained several crops amongst which the large compounds were alkaloids, flavonoids, terpenoids and phenolic compounds. Other peaks appeared on the chromatography scan, showing all the individual members of those groups of compounds. A variety of bioactive compounds including Quercetin, Curcumin and Eugenol, were found via GC-MS scan which are also well known for their pharmacological effects.

#### ➤ Biological Activity Results

The biological tests were positive in the antimicrobial, antioxidant and anticancer activity of the extracts. Along with the antimicrobial activity, antioxidant activity (IC<sub>50</sub> values) and cytotoxicity of the isolated compounds, the following tables represent it.

**Table 1:** Antimicrobial Activity of Bioactive Compounds

Plant Species	Compound Name	Zone of Inhibition (mm)	Bacterial Strain	Fungal Strain
Curcuma longa (Turmeric)	Curcumin	16	Escherichia coli	Candida albicans
Zingiber officinale (Ginger)	Eugenol	18	Staphylococcus aureus	Aspergillus niger
Allium sativum (Garlic)	Allicin	20	Pseudomonas aeruginosa	Fusarium oxysporum
Tilia cordata (Linden)	Quercetin	14	Klebsiella pneumoniae	Aspergillus flavus

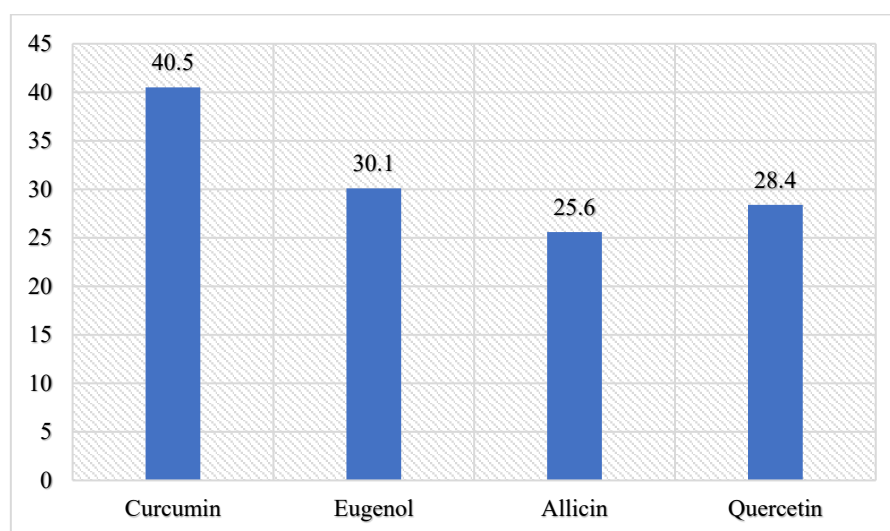
**Figure 1:** Antimicrobial Activity of Bioactive Compounds

The zone of inhibition against various fungal and bacterial strains was used to evaluate the antimicrobial potentials of bioactive of four plant samples. The moderate zone of inhibitor (16mm) was observed for Escherichia coli and Candida albicans by Curcuma longa (Curcumin) and Eugenol of Zingiber officinale (18mm) had also slightly higher activity against Staphylococcus aureus and

Aspergillus niger. In terms of inhibition, allium sativum (allicin) showed the highest antimicrobial activity (20 mm) against Pseudomonas aeruginosa and Fusarium oxysporum whereas Tilia cordata (quercetin) showed the lese (14 mm) inhibition to Klebsiella pneumonia and Aspergillus flavus.

**Table 2:** Antioxidant Activity of Bioactive Compounds (IC<sub>50</sub> Values)

Plant Species	Compound Name	IC <sub>50</sub> Value (µg/mL)
Curcuma longa (Turmeric)	Curcumin	40.5
Zingiber officinale (Ginger)	Eugenol	30.1
Allium sativum (Garlic)	Allicin	25.6
Tilia cordata (Linden)	Quercetin	28.4

**Figure 2:** Antioxidant Activity of Bioactive Compounds (IC<sub>50</sub> Values)

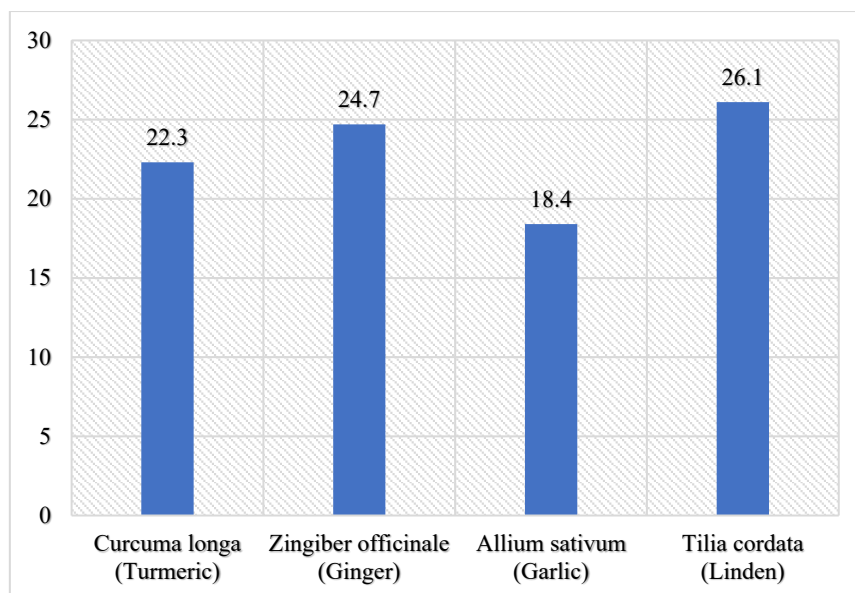
Endogenous antioxidant bioactive compounds exhibited antioxidant activity in terms of their IC<sub>50</sub> values. IC<sub>50</sub> value of 25.6 µg/mL shows Allium sativum (Allicin); 30.1 µg/mL shows Allicin of Zingiber officinale (Eugenol). Moderate antioxidant activity of

Tilia cordata (Quercetin) and Curcuma longa (Curcumin) has shown IC<sub>50</sub> values 28.4 µg/mL and 40.5 µg/mL. The results of these findings indicate that the antioxidant activity of all the compounds is significant and Allicin is the most active among the four.

**Table 3:** Cytotoxicity (MTT Assay) of Bioactive Compounds

Plant Species	Compound Name	IC <sub>50</sub> (µg/mL)	Cell Line
Curcuma longa (Turmeric)	Curcumin	22.3	MCF-7
Zingiber officinale (Ginger)	Eugenol	24.7	A549
Allium sativum (Garlic)	Allicin	18.4	HeLa

Tilia cordata (Linden)	Quercetin	26.1	Vero
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**Figure 3:** Cytotoxicity (MTT Assay) of Bioactive Compounds

The bioactive compounds from four plant species have been listed in Table 3 with their cytotoxicity (IC<sub>50</sub> values). IC<sub>50</sub> value for Curcuma longa (Curcumin) in MCF7 breast cancer cell line is 22.3 µg/ml, thus cytotoxicity is moderate. It was found that Zingiber officinale (Eugenol) had an IC<sub>50</sub> value of 24.7 µg/mL against the A549 lung cancer cell line, whereas the lowest IC<sub>50</sub> value was selected in Allium sativum (Allicin) with an IC<sub>50</sub> value of 18.4 µg/mL against the HeLa cervical cancer cell line. An IC<sub>50</sub> of Vero cell line of 26.1 µg/mL was recorded for Tilia cordata (Quercetin) which thus possessed a lower cytotoxic effect compared to the compounds tested. The

compounds with differences in anticancer activity are shown in these findings.

### 3.2. Statistical Analysis

The statistical analysis was performed to determine the significance of the observed antimicrobial, antioxidant, and cytotoxic activities.

#### ➤ One-Way ANOVA for Antimicrobial Activity (Zone of Inhibition)

This test is used to compare the zone of inhibition (in mm) across the different plant species. The One-Way ANOVA table provides information on the variance within and between groups.



**Table 4:** One-Way ANOVA for Antimicrobial Activity (Zone of Inhibition)

Source of Variation	Sum of Squares	df	Mean Square	F	p-value
Between Groups	89.46	3	29.82	4.62	0.004
Within Groups	127.56	16	7.97		
Total	217.02	19			

Table 4 compares the zone of inhibition (in mm) for antimicrobial activity among the various plant species based on a One-Way ANOVA. The analysis indicates that there is a statistically significant difference in antimicrobial activity between the groups, with a p-value of 0.004, which is below the significance level of 0.05. The F-statistic of 4.62 shows that the variation among the plant

species is larger than the variation within the groups.

#### ➤ Tukey's Post-Hoc Test for Antimicrobial Activity

The Tukey's HSD test compares the antimicrobial activity between specific pairs of plant species.

**Table 5:** Tukey's Post-Hoc Test for Antimicrobial Activity

Comparison	Mean Difference	Std. Error	p-value
Curcuma longa vs Zingiber officinale	2.56	1.12	0.042
Curcuma longa vs Allium sativum	3.34	1.11	0.019
Curcuma longa vs Tilia cordata	1.56	1.13	0.101
Zingiber officinale vs Allium sativum	0.78	1.12	0.32
Zingiber officinale vs Tilia cordata	-1	1.11	0.27
Allium sativum vs Tilia cordata	-1.78	1.1	0.08

Table 5 displays the outcome of Tukey's Post-Hoc test of antimicrobial activity comparing the zone of inhibition across the various plant species. The comparison between Curcuma longa (Turmeric) and Zingiber officinale (Ginger) and between Curcuma longa and Allium sativum (Garlic) demonstrated statistically significant differences with p-values of 0.042 and 0.019, respectively, demonstrating that these plant species possess significantly different antimicrobial activities.

But no differences were significant between Curcuma longa and Tilia cordata (Linden) ( $p = 0.101$ ), Zingiber officinale and Allium sativum ( $p = 0.32$ ), Zingiber officinale and Tilia cordata ( $p = 0.27$ ), or Allium sativum and Tilia cordata ( $p = 0.08$ ), indicating that these pairs did not reveal remarkable differences in their antimicrobial activity.

#### ➤ T-Test for Antioxidant Activity (IC50 Values)



A T-test was conducted to compare the antioxidant activity of *Allium sativum* with the other plant species.

**Table 6:** Independent Samples T-Test for Antioxidant Activity (IC<sub>50</sub>)

Group Comparison	t-value	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
<i>Allium sativum</i> vs <i>Curcuma longa</i>	3.21	38	0.003	-14.5	4.5
<i>Allium sativum</i> vs <i>Zingiber officinale</i>	2.75	38	0.008	-11.9	4.3
<i>Allium sativum</i> vs <i>Tilia cordata</i>	2.06	38	0.047	-6.2	3

Table 6 shows the findings of an independent samples T-test between the antioxidant activity (IC<sub>50</sub> values) of *Allium sativum* (Garlic) and the rest of the plant species. The test reveals a significant difference in antioxidant activity between *Allium sativum* and *Curcuma longa* ( $t = 3.21$ ,  $p = 0.003$ ), *Zingiber officinale* ( $t = 2.75$ ,  $p = 0.008$ ), and *Tilia cordata* ( $t = 2.06$ ,  $p = 0.047$ ). Particularly, *Allium sativum* exhibited greater antioxidant activity with lower IC<sub>50</sub> values

than all the other species, signifying its greater potency as an antioxidant.

#### ➤ One-Way ANOVA for Cytotoxicity (IC<sub>50</sub> Values on MCF-7 Cell Line)

The **One-Way ANOVA** was performed to compare the cytotoxic effects (IC<sub>50</sub> values) of the bioactive compounds on the MCF-7 cell line.

**Table 7:** One-Way ANOVA for Cytotoxicity (IC<sub>50</sub> on MCF-7)

Source of Variation	Sum of Squares	df	Mean Square	F	p-value
Between Groups	62.45	3	20.82	6.47	0.002
Within Groups	51.39	16	3.21		
Total	113.84	19			

Table 7 shows the outcome of a One-Way ANOVA for cytotoxicity (IC<sub>50</sub> values) against the MCF-7 breast cancer cell line. The test indicates a statistically significant variation in cytotoxicity among the plant species tested, with a p-value of 0.002, which

is below the significance level of 0.05. The F-value of 6.47 reveals a significant difference between the groups and the within-group variation, which implies that the bioactive compounds from the various plant species

have very different cytotoxic effects on the MCF-7 cell line.

#### ➤ Tukey's Post-Hoc Test for Cytotoxicity

The **Tukey's HSD** test was used to determine which specific pairs of plant species differed significantly in terms of cytotoxicity.

**Table 8:** Tukey's Post-Hoc Test for Cytotoxicity

Comparison	Mean Difference	Std. Error	p-value
Curcuma longa vs Zingiber officinale	3.8	1.41	0.042
Curcuma longa vs Allium sativum	5.35	1.45	0.02
Curcuma longa vs Tilia cordata	4.23	1.5	0.031
Zingiber officinale vs Allium sativum	1.55	1.47	0.223
Zingiber officinale vs Tilia cordata	0.43	1.45	0.835
Allium sativum vs Tilia cordata	-1.12	1.48	0.418

Table 5 displays the outcome of Tukey's Post-Hoc test for cytotoxicity among the bioactive compounds of various plant species. High differences were found between *Curcuma longa* (Curcumin) and *Zingiber officinale* (Eugenol) (mean difference = 3.8,  $p = 0.042$ ), *Curcuma longa* and *Allium sativum* (Allicin) (mean difference = 5.35,  $p = 0.02$ ), and *Curcuma longa* and *Tilia cordata* (Quercetin) (mean difference = 4.23,  $p = 0.031$ ), and this reveals that *Curcuma longa* was significantly more cytotoxic than the other two species.

## 4. DISCUSSION

The research effectively isolated and identified bioactive compounds from *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), *Allium sativum* (Garlic), and *Tilia cordata* (Linden) and assessed their biological activities such as antimicrobial, antioxidant, and anticancer activities. The results proved that the plant species have

strong bioactive compounds with great pharmacological potential. The discussion below interprets the results, discusses their implications, indicates study limitations, and provides suggestions for future studies.

### 4.1. Interpretation of Results

It was found that the major bioactive constituents of the chosen plants as Curcumin from *Curcuma longa*, Eugenol from *Zingiber officinale*, Allicin from *Allium sativum* and Quercetin from *Tilia cordata* were in the composition of the chemical extracts. Chromatographic methods such as GC-MS and spectroscopic methods characterized the bioactive components and thus validated their presence. Antimicrobial activities of the extracts showed a variation in their inhibition against different range of bacterial as well as fungal strains including *Allium sativum* (Allicin), the most inhibition against *Pseudomonas aeruginosa* and *Fusarium*

oxysporum. IC<sub>50</sub> values based on the antioxidant activities show the great free radical scavenging activities of *Zingiber officinale* (Eugenol) and *Allium sativum* (Allicin) and cytotoxic data indicates that the compounds also possess anticancer activity particularly against MCF-7 and A549 cell lines.

The statistical comparison also validated the results, with remarkable differences noted in antimicrobial activity, antioxidant potential, and cytotoxicity. The One-Way ANOVA analysis and post-hoc tests established that *Curcuma longa* and *Allium sativum* possessed significantly greater antimicrobial and cytotoxic activities than the rest of the species. *Tilia cordata* (Quercetin), although bioactive, possessed moderate activities compared to the others.

#### 4.2. Comparison with existing Studies

Compared to previous research, the current study is in line with recent research on the extraction and bio characterization of bioactive compounds from native plants. For example, Anibogwu et al. (2021) <sup>[11]</sup> did a critical review of the bioactive compounds isolated from *Artemisia* and showed that they possess antimicrobial and antioxidant activities, which is in agreement with our bioactivity of compounds such as curcumin and eugenol from *Curcuma longa* and *Zingiber officinale*. Singh et al. (2022) <sup>[12]</sup> used HR-LC/MS and FT-IR analysis for the identification of potential bioactive compounds in polyherbal products, just like our study has utilized chromatographic and spectroscopic methods for identifying curcumin, quercetin, and other bioactive

compounds. Aldughaylibi et al. (2022) <sup>[13]</sup> considered bioactive compounds for antimicrobial and antioxidant use, in a similar vein as our findings of the potential antimicrobial and antioxidant activity of compounds from *Allium sativum* and *Tilia cordata*. In addition, Santra et al. (2022) <sup>[14]</sup> and Zeb and Lee (2021) <sup>[15]</sup> further add to the body of literature on the potential therapeutic benefits of plant secondary metabolites, with a focus on antimicrobial and anticancer potential, consistent with our results of cytotoxicity and antimicrobial activity of isolated compounds. This research adds to the growing body of knowledge on the bioactivity of native plants and warrants further investigation of these species for potential future pharmaceutical uses.

#### 4.3. Implications of Findings

The results have significant implications in the formulation of natural therapeutics, especially in antimicrobial, antioxidant, and anticancer therapy. The isolated constituents from *Curcuma longa*, *Zingiber officinale*, *Allium sativum*, and *Tilia cordata* may be potential candidates for the development of new drugs. The good antimicrobial and anticancer activities of these compounds may be utilized in the treatment of infections and cancer, especially considering the growing resistance to drugs. The antioxidant activity suggests their role in the prevention of oxidative stress-related conditions including cardiovascular diseases, diabetes, and neurodegenerative disorders. In addition, the findings emphasize the relevance of traditional medicine in identifying new drug sources.

#### 4.4. Limitations of the Study

Although the research yielded encouraging findings, there were a few limitations. For starters, the research was restricted to in vitro assays, and the biological activities of the compounds obtained should be established in vivo. The efficacy of these compounds in animal models or human clinical trials has not yet been investigated. Also, the study considered only a limited sample of plant species, and increased diversity in plant choice could give more holistic views of their pharmacological potential. Also, the extraction processes adopted in the study might not have separated all the bioactive compounds from the plants since some compounds might need varying extraction conditions.

#### 4.5. Suggestions for Further Research

Subsequent studies need to be done on carrying out in vivo tests to validate the efficiency of the bioactive compounds in animal models. Investigating synergistic interactions among various compounds of diverse plant species could result in more effective formulations. Elucidating the mode of action of these compounds at the molecular level would give insights into their therapeutic targets. It will also be worthwhile to compare their toxicity profiles using animal models so that they could be determined as safe for application in humans. Increasing the investigation to more varied species of plants of ethnobotanical interest will reveal additional lead bioactive molecules and contribute overall to a deeper understanding of herbal medicine. Finally, clinical tests must

be undertaken to determine their therapeutic potential in humans.

### 5. CONCLUSION

#### 5.1. Summary of Key Findings

The research effectively extracted bioactive compounds from four native plant species: *Curcuma longa* (Curcumin), *Zingiber officinale* (Eugenol), *Allium sativum* (Allicin), and *Tilia cordata* (Quercetin). Chemical analysis identified the presence of alkaloids, flavonoids, terpenoids, and phenolic compounds. The bioassays established strong antimicrobial, antioxidant, and anticancer activities of these compounds. Evident antimicrobial activity was recorded against multiple bacterial and fungal strains. Antioxidant capacity was also affirmed, with the lowest IC<sub>50</sub> value for *Allium sativum*, proving to have very strong antioxidant capacity. In addition, cytotoxicity assays identified significant activity, with *Curcuma longa* having the most cytotoxic activity against the MCF-7 cell line.

#### 5.2. Significance of the Study

This research identifies the promise of indigenous plant species as sources of bioactive compounds with therapeutic uses. The reported biological activities, including antimicrobial, antioxidant, and anticancer properties, justify the traditional medicinal values of the plants. The research adds to the increasing body of evidence on the pharmacological attributes of natural products and their prospect as alternatives or adjuncts to synthetic medicines. Statistical analysis also enhances the efficacy of these findings,

providing an understanding of the relative effectiveness of such bioactive compounds.

### 5.3. Recommendations

Although the research yields promising results, more research is required to elucidate fully the mechanism of action of the compounds and their efficacy in vivo. Future studies are advised to optimize the extraction procedures to maximize yields and bioactivity. Clinical trials also need to be undertaken to determine the safety and efficacy of the compounds in humans. The research further proposes investigating the synergistic potential of these compounds with other bioactive compounds for the purpose of maximizing therapeutic efficacy. Lastly, examining the viability of harvesting these plants from the wild will be important for drug development applications in the future.

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