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Research Article

# An Assessment of Phytochemical, Anti-microbial, and Anti-oxidant Properties of *Tinospora sinensis* and *Berberis aristata* of Nepal

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## Abstract

Traditional medicine and the use of indigenous medicinal plants have been an integral part of the healthcare system and cultural practices in Nepal for generations. However, there has been a lack of systematic research on their antioxidant and antimicrobial properties. Our study focused on evaluating the medicinal potential of two indigenous plants of Nepal; *Tinospora sinensis* and *Berberis aristat*. The plant samples were collected from different ecological regions in Nepal, specifically *Tinospora sinensis* and *Berberis aristat*, from altitudes of 1161 and 1666m, respectively, in the hills of Dhunibesi-7, Dhading, Nepal. Crude methanolic extracts were prepared from these plants using the Soxhlet apparatus method and concentrated through rotary vacuum evaporation. Preliminary phytochemical analysis revealed the presence of important compounds such as alkaloids, carbohydrates, tannins, flavonoids, saponins, resins, proteins, amino acids, and fatty acids. Antimicrobial efficacy was assessed using Agar-disc diffusion against gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and gram-negative (*Klebsiella pneumoniae, Enterobacter*) bacteria. All extracts exhibited antimicrobial activity, with B. aristata leaves being most effective against B. subtilis. The antioxidant potential was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, with B. aristata stem extract showing the strongest activity (IC50: 82.101 µg/ml) and T. sinensis leaf extract showing the lowest (IC50: 128.53 µg/ml). Notably, both *Tinospora sinensis* and *Berberis aristat* leaves demonstrated superior antioxidant performance. In conclusion, *Tinospora sinensis* and *Berberis aristat* emerge as promising sources of medicinal compounds. Their diverse phytochemical composition likely contributes to their observed antimicrobial and antioxidant properties. This study emphasizes the value of these indigenous plants in Nepal's traditional medicine and highlights their potential as sources of medicinal and antioxidant agents.

**Keywords:** Anti-microbial Activity; Anti-oxidant Activity; Phytochemicals; Zone of Inhibition; Half Maximal Inhibitory Concentration ($IC_{50}$)

## Introduction

Nepal's unique geographical and climatic diversity has fostered the growth of a remarkable variety of flora, including an estimated 6,500 species of medicinal plants [1]. These plants have played an indispensable role in the traditional healthcare systems of various ethnic communities across Nepal [2]. The country's cultural and ecological diversity has led to the accumulation of indigenous knowledge about the therapeutic uses of these plants, passed down through generations [3].

Medicinal plants constitute a fundamental element of healthcare in Nepal. They are utilized to treat a spectrum of ailments, ranging from common colds to chronic diseases like diabetes and cardiovascular disorders [3]. The traditional healthcare practices rooted in indigenous knowledge have enabled communities, particularly those residing in rural and remote areas with limited access to modern medical facilities, to manage their health effectively.

In recent years, there has been growing interest in exploring the potential of Nepal's medicinal plants for modern medicine. Many of these plants contain bioactive compounds that exhibit pharmacological activities, including antimicrobial, anti-inflammatory, and anticancer properties [4]. The integration of these plants into mainstream healthcare systems could offer safer, more affordable, and locally accessible treatment options.

Despite the evident potential of Nepal's medicinal plants, a substantial gap exists in terms of systematic research. Limited funding, inadequate infrastructure, and a lack of interdisciplinary collaboration have hindered comprehensive investigations into the pharmacological properties, safety profiles, and optimal usage of these plants [1]. The scarcity of well-documented scientific data has impeded their inclusion in evidence-based medical practices.

Nepal's extensive medicinal plant resources offer a promising avenue for propelling modern medicine and improving healthcare access, especially for marginalized communities. The intertwining of traditional wisdom and modern science can greatly enhance healthcare outcomes. However, to fully tap into this potential, substantial investments in comprehensive research, knowledge sharing, and collaboration are imperative.

## Materials and Methods

### Plant collection and identification

The stem and leaf samples of *T. sinensis* and *B. aristata* were collected from an altitude of 1161m and 1666m from the hills of Dhunibesi-7, Dhading, by the method of 'Quadrant sampling' using sterile tools and techniques. The samples were taxonomically identified by the National Herbarium and Plant Laboratory, Godavari, Lalitpur, Nepal.

### Preparation of plant extract

The plant samples were cut into pieces and dried under shade in a room with proper ventilation at room temperature for 2-3 weeks. The dried samples were pulverized into powder using a mechanical grinder. 100 gm of each powdered sample was placed in a Whatman cellulose thimble and was extracted in the Soxhlet extraction system using 750 ml of methanol for 72 h. The crude extract solutions were then fully dried using a rotary vacuum evaporator at a temperature of 60°C to remove the solvent. High temperature was avoided to minimize the component degradation. The samples were then stored in a refrigerator at 4°C [5].

### Proximate value analysis

Proximate value analysis was conducted to gain insights into the physical and chemical characteristics of the plant extracts, which are indicative of their stability and potential applications [6]. Specifically, the analysis focused on the total moisture content and the total ash percentage of the extracts.

### Total moisture content

1 gram of powdered plant extract was taken in a Petri plate and kept in a hot air oven at 105°C for about 30 minutes, and then the weight was taken. This process was continued till the constant weight was obtained. The final weight of the powder was noted, and loss on drying was calculated [6,7]. The total moisture content was calculated using the formula:

Total Moisture Content = (Loss on Drying/Fresh Weight) * 100%

### Total ash value

A clean silica crucible was weighed and dried in the hot air oven for a few minutes. About 1 gram of the powdered plant extract was weighed and placed in the silica crucible. A fine layer of powdered

plant extract was spread uniformly on the silica crucible. The crucible was kept inside the muffle furnace for 6 hours at 450°C to make the crucible dull red hot until free from carbon. The Crucible was allowed to cool down at room temperature and weighed. The same procedure was repeated till constant weight. The percentage of total ash was calculated and noted down [6,7]. The total ash percentage was calculated by the formula:

Total ash percentage = (Ash Weight/Fresh Weight) * 100%

### Phytochemical screening

A qualitative phytochemical screening was conducted to ascertain the primary classes of chemical compounds present in the crude stem and leaf extracts of *T. sinensis* and *B. aristata*. A few milligrams of the obtained crude extracts were taken and dissolved in a conical flask containing about 100 ml of DMSO separately. These were considered as the 'test solution' for the preliminary phytochemical analysis. The following procedure was carried out for each of the plant extract samples.

### Test for flavonoid

### Alkaline reagent test

The test solution was treated with a few drops of sodium hydroxide solution giving off an intense yellow color, which turned colorless on the addition of diluted. Acetic acid. This indicated the presence of flavonoids [8].

### Zinc-hydrochloride reduction test

A mixture of 5 ml of dil. ammonia and conc. sulphuric acid was added to the test solution giving off a red color indicating the presence of flavonoid [8,9].

### Shinoda test

The test solution was diluted with ethanol, and a few pieces of magnesium chips, followed by the addition of conc. hydrochloric acid. The development of a pink color indicated the flavonoid's presence [8,9].

### Lead acetate, sulphuric acid and ammonia test

The addition of a few ml of lead acetate solution, conc. sulphuric acid and ammonia to the test solution formed a yellow precipitate, orange, and yellow color, respectively, which was indicative of the presence of flavonoids [8,9].

### Test for alkaloids

A few ml of test solution was taken in a clean test tube and treated with Mayer's reagent (potassium mercuric iodide solution), Wagner's reagent (iodine and potassium iodide solution), and Hager's reagent (saturated solution of picric acid). The cream, brown flocculent, and orange-yellow precipitate obtained with Mayer's reagent, Wagner's reagent, and Hager's reagent, respectively, indicated the presence of alkaloids [8,9].

### Test for carbohydrate

Treatment of a small amount of the test solution dissolved in 5 ml of distilled water with Molisch's reagent (alcoholic alpha-naphthol) along with 1 ml of conc, sulphuric acid, Fehling's solution A and B, and Benedict's reagent (mixture of sodium citrate, sodium carbonate, and the pentahydrate of copper (II) sulfate) gives a reddish brown ring at the common surface of the liquids, a red precipitate of cuprous oxide, and a reddish brown precipitate. This observation validated the presence of carbohydrates [8,9].

### Test for tannin

The observation of the dark green or deep blue color and white precipitate in the test solution when treated with ferric chloride reagent (5% w/v solution of ferric chloride prepared in 90% ethanol) and 1% gelatin solution containing 10% sodium chloride indicated the presence of tannin [8-10].

### Test for Saponin

A portion of the test solution was shaken vigorously with sodium bicarbonate and distilled water. A stable, characteristic honeycomb-like froth was obtained, validating the presence of saponin [8-10].

### Test for sterol

2 ml of chloroform and 2 ml of conc. sulphuric acid was added to the test solution, followed by shaking for a few minutes. The development of red color in t the layer of chloroform validated the presence of sterol [8-10].

### Test for fatty acid and fixed oil

A small quantity of plant extract was pressed between two filter papers and allowed to dry at room temperature. The stain on the first filter paper indicated the presence of fixed oils [8-10].

### Test for resin

A small amount of test solution was treated with a few ml of acetone and shaken. The appearance of turbidity confirmed the presence of resin [8-10].

### Test for amino acids

The test solution was treated with a few ml of 0.25% ninhydrin solution and 1 ml of 10% sodium hydroxide solution along with a drop of 0.7% copper sulfate solution separately and heated in a water bath. The formation of a blue-violet color confirmed the presence of amino acids [8-10].

### Antimicrobial assay

Kirby-Bauer disk diffusion method was used to assess the antimicrobial effect of all the methanolic extracts of *T. sinensis and B. aristata* against the ATCC (American Type Cell Culture) of the test organisms: gram-positive (*Bacillus subtilis* and *Staphylococcus aureus)* and gram-negative *(Klebsiella pneumoniae, and Enterobacter).* The viability of each bacterium was tested by culturing them in nutrient broth, followed by sub-culturing on nutrient agar medium under sterile conditions with the incubation condition of 37˚C. Fresh bacterial cultures were then produced from this stock culture and cultivated in nutrient broth for 24 h at 37˚C. The final counts of bacteria were adjusted to a 0.5 McFarland turbidity standard, which corresponds to approximately 1.5 × 10^8 colony-forming units (CFU) per milliliter [11]. Sterile disks of 6mm made using grade 1 Whatman filter paper were immersed in the four concentrations of each extract prepared in 0.2% DMSO: 50mg/ml, 100mg/ml, 200mg/ml, and 400mg/ml for 5 min. The immersed disks were deposited on Mueller Hinton agar plates containing the test organisms that had been previously inoculated and incubated at 37˚C [12]. The diameter of the zone of inhibition (mm) was measured every 4 h till 12 h. 0.2% DMSO and gentamicin was used as negative and positive controls, respectively, in the same manner as a plant extract [13].

### Antioxidant assay

The antioxidant potential of each plant extract was evaluated using the DPPH scavenging free radical assay. A solution of DPPH at 0.1 mM concentration was created by dissolving 3.9 mg of DPPH in 100 mL of methanol. This purple DPPH solution was prepared at 4°C and stored at -20°C [14].

For the assessment of antioxidant activity, different concentrations (10, 25, 50, 75, and 100 µg/mL) of methanolic solutions of each extract were prepared by serial dilution from the stock solution of the respective extract. To each 0.5 mL of the extract solution, 2.5 mL of the 0.1 mM methanolic DPPH solution was added. A control was established using 0.5 mL of distilled water with 2.5 mL of the 0.1 mM methanolic DPPH solution. After thorough shaking, the samples were incubated in darkness at room temperature for 30 minutes.

Absorbance readings at 517 nm were taken against a blank solution consisting of 2.5 mL methanol and 0.5 mL distilled water. The radical scavenging activity was expressed as the percentage of scavenged radicals using the equation where, As is the absorbance of the sample solution, Ab is the absorbance of the blank, and Ac is the absorbance of the control [15].

% Scavenging Activity =

The experiment was carried out in triplicate, and the $IC_{50}$ value, which represents the concentration of the sample required to scavenge 50% of the DPPH free radicals, was determined by plotting the radical scavenging activity against the concentration of the extracts. A lower IC50 value, the higher DPPH scavenging ability, i.e., higher antioxidant activity [14-16].

### Data analysis

All the experiments were carried out in triplicates, and results were represented as mean ± standard deviation. For antimicrobial data, statistical significance between the groups was analyzed using a two-tailed Student's t-test in Microsoft Excel. For antioxidant assay, calculation of linear correlation coefficient and correlation analysis were carried out. The linear regression equation for a straight line is Y = mx + c where Y = absorbance of extract, m = slope of the calibration curve, x = concentration of extract, and c = intercept. Using this regression equation, concentrations of extracts were calculated.

## Results and Discussion

### Proximate analysis

The proximate value analysis of the four plant extracts focused on their moisture content and ash percentage, revealing important insights into their stability and potential uses. Moisture content refers to the water present in a substance [7]. The study found that the stem extract of *B. aristata* had the lowest moisture content at 8.5%, while the leaf extract of *T. sinensis* had the highest at 12.5%. This suggests that the stem extract contains less water, which is beneficial for preventing degradation and microbial growth over time. Ash percentage indicates the mineral content in extracts [6]. The leaf extract of *B. aristata* had the lowest ash percentage (4.99%), while the stem extract had the highest (9.01%) [38]. Higher ash content can signify greater mineral presence, possibly impacting nutritional value or other applications. The findings suggest that the stem and leaf extracts of *B. aristata* possess unique qualities contributing to their physical and chemical stability. Lower moisture content in the stem extract indicates resistance to degradation, and higher ash content in the same extract implies potential mineral benefits (Table 1,2) [6,7].

These results have practical implications, aiding decisions in the storage, formulation, and application of extracts. The analysis contributes to understanding the plant extracts, guiding their utilization in various industries.

**Table 1:** Total moisture content of stem and leaf extracts of *T. sinensis* and *B. arista.*

| Plant Sample | Fresh Weight (gm) | Dry Weight (gm) | Loss on Drying (gm) | Loss on Drying (%) |
|---|---|---|---|---|
| *B. aristata* stem | 2.0 | 1.83 | 0.17 | 8.5 |
| *B. aristata* leaf | 2.0 | 1.77 | 0.23 | 11.5 |
| *T. sinensis* stem | 2.0 | 1.81 | 0.19 | 9.25 |
| *T. sinensis* leaf | 2.0 | 1.75 | 0.25 | 12.5 |

**Table 2:** Total ash value of stem and leaf extracts of *T. sinensis* and *B. arista.*

| Plant Sample | Fresh Weight (gm) | Ash Weight (gm) | Total Ash(%) |
|---|---|---|---|
| *B. aristata* stem | 3.0 | 0.27 | 9.01 |
| *B. aristata* leaf | 3.0 | 0.15 | 4.99 |
| *T. sinensis* stem | 3.0 | 0.25 | 8.32 |
| *T. sinensis* leaf | 3.0 | 0.16 | 5.31 |

### Preliminary phytochemical screening

Phytochemical analysis of the methanolic extract from both the stem and leaf of *T. sinensis* and *B. aristata* revealed the presence of various categories of bioactive compounds. The outcomes of this investigation have been organized and presented in the tables below (Tables 3 and 4).

### Antimicrobial activity

The antimicrobial properties of plants in combating infections make them highly intriguing in their potential therapeutic applications [18]. The present study investigated the antimicrobial activity of methanolic extracts obtained from the stems and leaves of *T. sinensis* and *B. aristata* at different concentrations (50, 100, 200, and 400 mg/ml) against gram-positive (*B. subtilis* and *S. aureus*) and gram-negative (*K. pneumoniae* and *Enterobacter*) bacteria. The results demonstrated that the antimicrobial activity was concentration-dependent (Figure 1-4), implying that higher extract concentrations were associated with stronger inhibitory effects against the tested bacteria [19]. The concept of dose-dependent responses, which states that increased concentrations of antimicrobial agents result in enhanced bacterial inhibition, supports this observation. This pattern highlights the potential of these extracts for therapeutic use, as higher concentrations may produce more pronounced effects [18,19].

| Compounds | Tests Performed | Results |
|---|---|---|
| Flavonoids | Alkaline reagent test | + |
| | Zinc-hydrochloride reduction test | + |
| | Shinoda test | + |
| | Lead acetate test | + |
| | Sulphuric acid test | + |
| | Ammonia test | + |
| Alkaloids | Mayer's reagent test | + |
| | Wagner's reagent test | + |
| | Hager's reagent test | + |
| Carbohydrates | Molisch's test | + |
| | Fehling's test | + |
| | Benedict's reagent test | + |
| Tannin | Ferric Chloride test | + |
| | Gelatin test | + |
| Saponin | Foam test | + |
| Sterol | Salkowaski reaction test | - |
| Fats & Oils | Spot test | - |
| Resin | Acetone-water test | - |
| Amino Acids | Ninhydrin test | + |
| | Biuret test | + |
| '+' = presence and '-' = Absence | | |

**Table 3:** Phytochemical screening of stem extracts of *T. sinensis* and *B. aristate.*

| Compounds | Tests Performed | Results |
|---|---|---|
| Flavonoids | Alkaline reagent test | + |
| | Zinc-hydrochloride reduction test | + |
| | Shinoda test | + |
| | Lead acetate test | + |
| | Sulphuric acid test | + |
| | Ammonia test | + |
| Alkaloids | Mayer's reagent test | + |
| | Wagner's reagent test | + |
| | Hager's reagent test | + |
| Carbohydrates | Molisch's test | + |
| | Fehling's test | + |
| | Benedict's reagent test | + |
| Tannin | Ferric Chloride test | + |
| | Gelatin test | + |
| Saponin | Foam test | + |
| Sterol | Salkowaski reaction test | - |
| Fats & Oils | Spot test | - |
| Resin | Acetone-water test | + |
| Amino Acids | Ninhydrin test | - |
| | Biuret test | - |
| '+' = presence and '-' = Absence | | |

**Table 4:** Phytochemical screening of leaf extracts of *T. sinensis* and *B. aristate.*

Among the tested extracts, the stem extract of *B. aristata* demonstrated the most remarkable antimicrobial efficacy, particularly against *B. subtilis.* The inhibition zone was substantial, reaching 15.75 mm at a concentration of 400 mg/ml. This potent activity aligns with previous research illustrating that *B. aristata* has antibacterial properties against various pathogens. Berberine, an alkaloid present in the plant, has been associated with antibacterial effects and is often credited for its ability to disrupt bacterial membranes and inhibit growth [20,21].

Interestingly, the susceptibility of different bacteria varied based on the type and concentration of plant extract. For instance, *K. pneumoniae* exhibited relatively lower susceptibility to the stem extracts of both *B. aristata* and *T. sinensis*, as well as the leaf extract of *T. sinensis*. Such variations in susceptibility are frequently observed and can be attributed to differences in bacterial cell wall structure and membrane properties [18].

## Antioxidant activity

The evaluation of antioxidant activity through the DPPH assay is a crucial approach for assessing the potential health benefits of natural extracts. This assay measures the ability of compounds to scavenge free radicals and prevent oxidative damage [16,19]. In this study, absorbance measurements were taken at a wavelength of 517 nm across varying concentrations (10, 25, 50, 75, and 100µg/

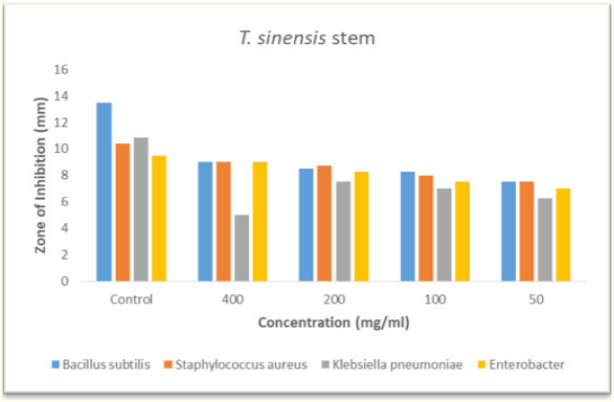


**Figure 1:** Antimicrobial effect of methanolic stem extract of *T. sinensis.*

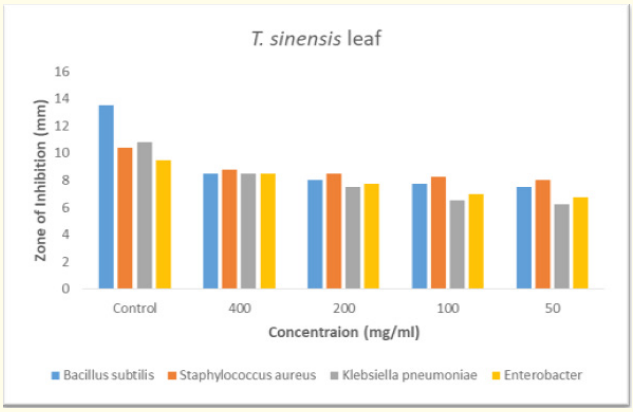


**Figure 2:** Antimicrobial effect of methanolic leaf extract of *T. sinensis.*

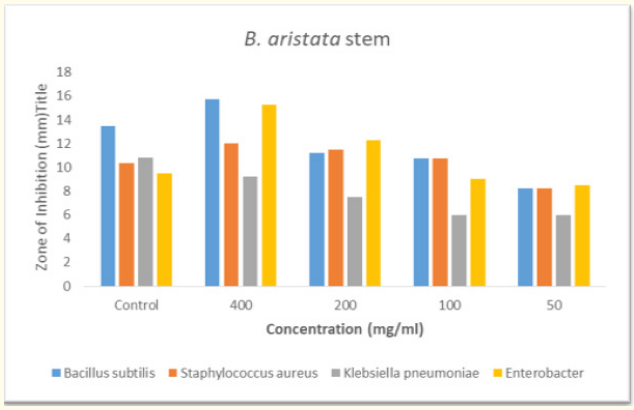


**Figure 3:** Antimicrobial effect of methanolic stem extract of *B. aristate.*

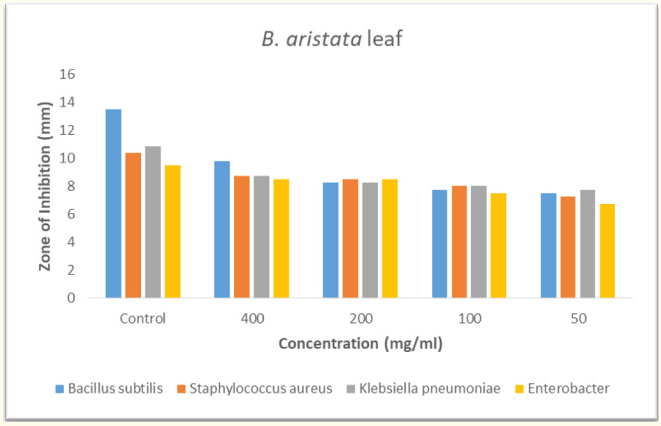


**Figure 4:** Antimicrobial effect of methanolic leaf extract of *B. aristate.*

ml) of extracts and L-Ascorbic acid (control). These readings were then utilized to compute the percentage of DPPH radical inhibition by the samples (Figure 5).

The investigations unveiled a concentration-dependent trend in the DPPH scavenging activity of various extracts, with efficacy increasing with an increase in the concentration of extracts. This pattern supports the idea that higher concentrations of antioxidants yield greater radical scavenging potential. The $IC_{50}$ values, representing the concentration at which 50% inhibition occurred, were derived from the percentage inhibition values at different concentrations, as depicted in (Figure 6). The IC50 values were 115.47. 128.5, 82.10, and 96.15 µg/ml for stem and leaf extracts of *T. sinensis* and *B. aristata*. Among the four extracts, the stem extract of *B. aristata* showed the strongest antioxidant activity, with an IC50 value that is the lowest and closest to the control L-ascorbic acid (25.38 µg/ml). The finding that *B. aristata* stem extract exhibited the strongest antioxidant activity is consistent with previous research on its bioactive components, such as berberine, which has shown significant free radical scavenging abilities [21].

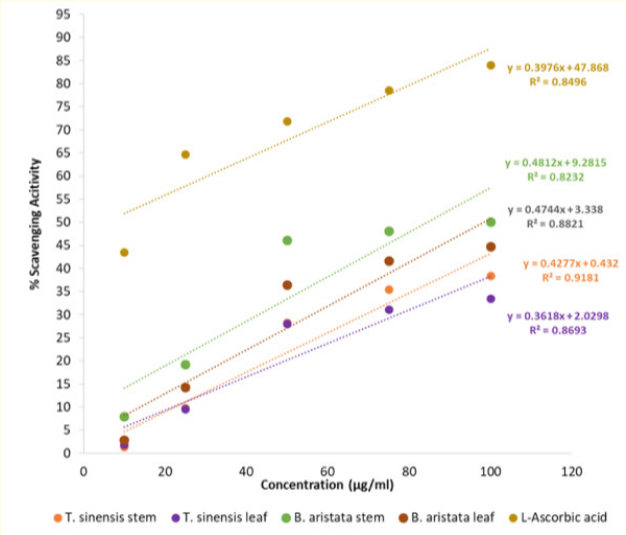


**Figure 5:** Concentration-dependent DPPH radical scavenging activity of extracts.

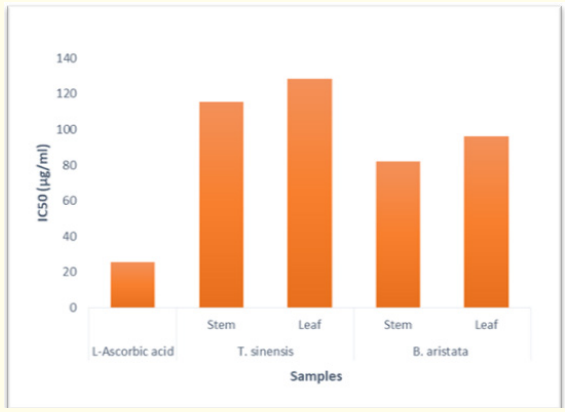


**Figure 6:** $IC_{50}$ values of plant extracts and L-Ascorbic acid (control).

## Conclusion

The comprehensive analysis of stem and leaf extracts of *T. sinensis* and *B. aristata,* including their proximate value, phytochemical composition, and antimicrobial and antioxidant activities, has provided valuable insights into their potential therapeutic applications. The stem and leaf extracts of *B. aristata* displayed distinct qualities, with lower moisture content indicating enhanced stability and higher ash content suggesting potential mineral benefits. The concentration-dependent antimicrobial activity demonstrated by the extracts against both gram-positive and gram-negative bacteria highlights their therapeutic potential, particularly the potent efficacy of the stem extract of *B. aristata*. Additionally, the exceptional antioxidant activity observed in the extracts, evaluated through the DPPH assay, further emphasizes their potential health benefits. Notably, the stem extract of *B. aristata* exhibited the strongest antioxidant activity, reflecting its potential as a valuable source of free radical scavenging compounds. As we consider the implications of these findings, it is evident that both *B. aristata* and *T. sinensis*, with their distinct properties and bioactive compounds, hold great promise for applications in various industries and as natural sources of therapeutic agents.

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