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# Antioxidant and Antibacterial Activity of Alkaloid Fractions of *Tristemma hirtum* P. Beauv

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ABSTRACT

*Tristemma hirtum* is a medicinal plant used in Nigerian traditional medicine to treat various diseases. In this study, the *in vitro* antioxidant and antibacterial activity of alkaloid fractions from organs of *T. hirtum* was evaluated. The antioxidant activity of the alkaloid fractions was evaluated by measuring its 1,1-diphenyl-2-picryl hydrazyl (DPPH), hydrogen peroxide ($H_2O_2$), and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging activity. Also, the metal chelating and reducing power of the fractions was evaluated. Antibacterial activity was determined by the agar well diffusion method against clinical isolates of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonela typhi* and the minimum inhibitory concentration (MIC) evaluated by the broth microdilution technique. In the antioxidant assay, the alkaloid fractions exhibited significant antioxidant activity. The root alkaloid fraction exhibited pronounced DPPH, ABTS radical scavenging activity and metal chelating potential with $IC_{50}$ values of 19.34, 26.02 and 12.01 µg/mL, respectively. The stem alkaloid fraction exhibited high $H_2O_2$ scavenging activity ($IC_{50}$ = 17.46 µg/mL), while the leaf alkaloid fraction demonstrated the optimum reducing power ($IC_{50}$ = 20.04 µg/mL). The alkaloid fractions exerted remarkable antibacterial activity against the test organisms and MIC values ranged from 0.09 to 2.28 mg/mL, with *S. aureus* and *B. subtilis* as the most susceptible. The results highlighted that alkaloid fractions from *T. hirtum* may serve as natural sources of antioxidant and antibacterial leads and lend support to its use in traditional medicine.

***Keywords:*** *Tristemma hirtum*, alkaloid fractions, antioxidant activity, antimicrobial activity.

## Introduction

A wide range of ailments such as microbial infections and diseases associated with increased reactive oxygen species (ROS) in the body including inflammation, cardiovascular diseases, immune system dysfunction, arteriosclerosis, diabetes and neurogenerative diseases are being treated with medicinal plants in many cultures around the world.[1-2] Its use is very popular in Africa where the dependence on traditional medicine practice is still high. Despite the presence of synthetic drugs that can treat microbial infections and retard ROS, challenges such as the high cost, microbial resistance and toxicity have negatively impacted on the use of these synthetic drugs for effective health care delivery, resulting in treatment failures. This has prompted the renewed search for therapeutic alternatives from plant sources, which are considered to be safe, cost effective and affordable.[1]

*T. hirtum*, family Melastomataceae (local names: Orunchi, Igbo; Udia inuen, Ibibio; Apiko, Yoruba) is a medicinal plant that grows abundantly in Nigeria and in many West African countries. The leaves are used in traditional medicine to treat skin diseases, headache, menstrual pain and for blood purification. Limited biological activity has been reported for this plant. For instance, a decoction of the leaf extract showed antimicrobial, antidermatophytic and analgesic properties.[2-3] Also, flavonol glycosides, *β*-sitosterol glucopyranoside and terpenoid acids have been isolated from the ethyl acetate and n-butanol soluble fractions of the plant methanol extract.[4] In addition, phytocompounds such as alkaloids, triterpenes, steroids, flavonoids and polyphenols were identified in the methanol leaf extract of the plant.[3] However, numerous biological activities have been reported for other species of this family including antioxidant, antihypertensive, antimicrobial, cytotoxic, hemostatic, anti-inflammatory and antihyperglycemic properties.[4-6] Also, flavonoids, polyphenols, steroids, fatty acids, etc have been identified from members of this family.[7-10] As far as our literature search can ascertain, there is limited information on the bioactivity of these phytochemical from *T. hirtum* despite its traditional importance, thus we report in this study, the antioxidant and antibacterial activity of alkaloid fractions of *T. hirtum*.

## Materials and Methods

*Plant Material*

*T. hirtum* organs (leaf, stem and root) were collected in September 2019 from the wild in Nsit Ibom Local Government Area, Akwa Ibom State, and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria. The voucher specimen was deposited at the herbarium of the Department (voucher number UUH 813). The organs were dried at 40°C in an oven to constant weight. Dried organs were milled, sieved and stored in plastic containers prior to extraction.

Alkaloid Extraction

Dried and ground organs (leaf, stem and root) of *T. hirtum* (100 g each) was extracted with petroleum ether exhaustively in a Soxhlet apparatus. The defatted organs were then soaked in a basic aqueous

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methanol solution (methanol/water/$NaHCO_3$, 20:10:1), followed by extraction with dichloromethane in a Soxhlet apparatus. The resulting solution was filtered, concentrated in a rotary evaporator and washed with 5% HCl (4 x 50 mL). The slurry was adjusted to pH 8-9 with aqueous ammonia (25%) and re-extracted with dichloromethane (4 x 80 mL) and the organic phase filtered and concentrated to give the leaf, stem and root alkaloid fractions, respectively.[11] Extraction yields were 1.21%, 1.38% and 1.55% for the leaf, stem and root alkaloid fractions, respectively. The fractions tested positive to Dragendoff's reagent.

*Evaluation of Antioxidant activity*
The antioxidant activity of the alkaloid fractions was evaluated by measuring its DPPH, $ABTS^+$ radical, hydrogen peroxide scavenging, reducing power and metal chelating activities.

*Evaluation of DPPH radical scavenging activity*
Concisely, 1 mL of each fraction at varying concentrations (0-200 µg/mL) was mixed with1 mL of 0.004% methanol solution of DPPH. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature in the dark. The reduction of the DPPH radical was determined by measuring the absorbance at 517 nm. The procedure was repeated for the blank and control. The radical scavenging activity was calculated using the equation:

DPPH scavenging effect (%) = $[(A_{blank} – A_{sample})/A_{blank}] \times 100$.

Sample concentration providing fifty percent inhibition ($IC_{50}$) was calculated from the graph of inhibition percentage against extract concentration. Butylated hydroxyl anisole (BHA) was used as positive control.[12]

*Evaluation of ABTS radical scavenging activity*
ABTS radical was produced by reacting 7 mM ABTS solution (absorbance = 0.7 ± 0.02 at 734 nm) with 2.45 mM potassium persulfate. This mixture was allowed to stand for 12 hrs in the dark at room temperature. Then, 2.94 mL of this ABTS solution was mixed with 60 µL of each fraction and incubated at 37°C for 20 min in the dark. After incubation, the absorption was read at 734 nm. The percentage inhibition was calculated using the equation:

% inhibition = $[A_{blank} – A_{sample})/A_{blank}] \times 100$

Sample concentration providing fifty percent inhibition ($IC_{50}$) was calculated from the graph
of inhibition percentage against extract concentration. Vitamin C was used as positive control.[13]

*Evaluation of hydrogen peroxide scavenging activity*
Fractions of varying concentrations (0-200 µg/mL) (1 mL) was mixed with 400 µL of $H_2O_2$ (5 mM) in phosphate buffer (pH 7.4; 100 mM) and incubated for 20 min, after which the absorbance was read at 230 nm against a blank. Vitamin C was used as positive control.[14] The $H_2O_2$ inhibition (%) was determined using the equation:

$H_2O_2$ scavenging activity (%) = $[(A_{blank} – A_{sample})/A_{blank}] \times 100$.

*Evaluation of reducing power*
The reducing power was determined according to the method of Oyiazu, 1986.[15] Each fraction (10 -100 µg/mL) in ethanol (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.60), 2.5 mL of 1% potassium ferricyanide and the mixture incubated at 50°C for 20 min. Thereafter, 2.5 mL of 10% trichloroacetic acid (w/v) was added and the mixture centrifuged at 200 g for 19 min. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% ferric chloride and the absorbance measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power. $IC_{50}$ value (µg/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained from the regression equation. Vitamin C was used as positive control.

*Evaluation of Metal chelating activity*
Metal chelating activity was determined according to the method of Decker and Welch[16] with some modifications. Briefly, 0.5 mL of each fraction was mixed with 0.05 mL of 2 mM$FeCl_2$ and 0.1 mL of 5 mM ferrozine. The total volume was diluted with 2 mL methanol. Then, the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm. Ethylenediaminetetraacetic acid (EDTA) was used as a positive control. The percentage inhibition rate of ferrozine – $Fe^{2+}$ complex formation was calculated using the formula:

Scavenging activity (%) = $[(A_{control} – A_{sample})/A_{control}] \times 100$

where $A_{control}$ = absorbance of ferrozine – $Fe^{2+}$ complex, and $A_{sample}$ = absorbance of sample.

*Antibacterial activity*
Clinical isolates of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonela typhi* were used for this study. Each strain was sub-cultured overnight in Muller-Hilton agar (MHA) at 35°C. Bacterial suspensions were adjusted to $10^8$ cfu (colony forming unit)/mL by comparison with a 0.5 McFarland scale. The antimicrobial activity of the fractions was determined by the agar well diffusion method. The liquid bacterial cultures were spread on the agar surface, and a hole with a diameter of 6 mm was punched aseptically using a cork borer, packed with 40 µL of each fraction (10 µg/mL), allowed to settle and incubated for 24 hrs at 35°C. DMSO was used to prepare controls and the inhibition zone diameter (mm) measured.[17] Minimum inhibitory concentration (MIC) of the fractions was determined by the broth microdilution method in a 96-well microplate according to the guidelines of the Clinical and Laboratory Standards Institute.[18] MICs were recorded as the lowest concentration of the extract which showed bacterial growth inhibition. For minimum bactericidal concentration (MBC), 10 µL of suspension from the MIC was re-inoculated on MHA and incubated. MBC was defined as the lowest concentration that yielded negative subcultures.

*Statistical Analysis*
Experiments were repeated in triplicate. The data were subjected to analysis of variance (ANOVA) using Statistical Package for the Social Science (SPSS version 20.0, IBM Corp, USA). Levels of significance was maintained at 95% for each test.

## Results and Discussion

*Antioxidant activity*
Numerous findings agree that diseases such as cancer, diabetes, cardiovascular and neurodegenerative disorders are exacerbated by oxidative stress arising from the inability of the body to completely scavenge ROS, which damage biological molecules including lipids, proteins and deoxyribonucleic acid (DNA).[19-24] Despite the availability of synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT), there is increased search for natural antioxidants as these are considered to be safer, cheaper and less toxic. The antioxidant activity of alkaloid fractions of *T. hirtum* was evaluated by various *in-vitro* models including the DPPH, ABTS, $H_2O_2$ radical scavenging activity, reducing power and metal chelating activity. In the DPPH assay (Figure 1a), the root alkaloid fraction exhibited a stronger inhibitory effect than the stem and leaf fractions in a dose-dependent manner.
At 100 µg/mL, the root alkaloid fraction inhibited 81.15% of the DPPH radical, compared to 67.23% and 64.71% by the stem and leaf fractions, respectively. Similar results were obtained from $IC_{50}$ values (Table 1), with trend: root ($IC_{50}$ = 19.34 µg/mL) > stem ($IC_{50}$ = 44.35 µg/mL) > leaf ($IC_{50}$ = 58.83 µg/mL). However, the results were inferior to the positive control BHA ($IC_{50}$ = 10.07 µg/mL).
In comparison with other works, Maiza-Benabdesselam *et al.*[20] reported DPPH scavenging activity of 86.0% and 45.6% for alkaloid fractions of *F. bastardii* and *F. capreolata*, respectively at 50 µg/mL

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while Khamtache-Abderrahim *et al.*[21] reported an $IC_{50}$ value of 15.74 - 41.72 µg/mL for alkaloid extracts of *F. officinalis*. The results of this study demonstrated that the alkaloid fractions from *T. hirtum* were efficient DPPH scavengers, with the root alkaloid fraction as the most potent. The presence of hydrogen, availability of hydroxyl groups and degree of methylation influences the radical scavenging ability of alkaloids.[22,23] In the ABTS assay, the radical quenching ability of the alkaloid fractions increased in a dose-dependent manner (Figure 1b). At a concentration of 20 µg/mL, the leaf, stem and root alkaloid fractions scavenged 30.33%, 33.55% and 43.36% of the ABTS radical; this increased to 60.22%, 62.81% and 74.44%, respectively at 100 µg/mL. Overall, the root alkaloid fraction exhibited a higher ABTS scavenging ability ($IC_{50}$ = 26.02 µg/mL) than the stem and leaf fractions ($IC_{50}$ = 68.04 and 75.48 µg/mL, respectively (Table 1). However, these fractions demonstrated lower ABTS scavenging ability than the control, vitamin C ($IC_{50}$ = 13.50 µg/mL). Dalimunthe *et al.*[24] reported higher $IC_{50}$ values for alkaloid fractions of *L. cubeba*. Similarly, $H_2O_2$ scavenging activity also increased in a dose-dependent manner (Figure 2a), with the stem alkaloid fraction scavenging 55.66% and 80.42% of $H_2O_2$ at 20 µg/mL and 100 µg/mL, respectively. Indeed, the stem alkaloid fraction exhibited a higher $H_2O_2$ scavenging ability ($IC_{50}$ = 17.46 µg/mL) than the root and leaf fractions ($IC_{50}$ = 20.02 µg/mL and 78.86 µg/mL, respectively).

Figure 2b illustrates the reducing power of the alkaloid fractions. This test shows the ability of a compound or extract to break the free radical chain by hydrogen donation and serves as a significant indicator of potential antioxidant action.[25] The alkaloid fractions exhibited potent reducing power and this increased at higher concentration. In this study, the leaf alkaloid fraction showed a higher reducing power ($IC_{50}$ = 20.04 µg/mL) than the stem ($IC_{50}$ = 24.00 µg/mL) and root fractions ($IC_{50}$ = 38.41 µg/mL), suggesting an increased content of reductones capable of hydrogen donation. Lower reducing power was reported for alkaloid fractions of *F. officinalis*, *F. bastardii* and *F. cappreolata.*[20,21]

The ability of the alkaloid fractions to chelate transition metals, such as $Fe^{2+}$ was also evaluated. This test is important because $Fe^{2+}$ is regarded as a pro-oxidant that can accelerate lipid peroxidation by breaking down lipid peroxides and hydrogen to free radicals by the Fenton process.[26]

In this study, the fractions had effective $Fe^{2+}$ chelating capacity (Figure 3), although this increased in a dose-dependent manner. At 20 µg/mL, the leaf, stem and root alkaloid fractions chelated 56.44%, 67.43% and 75.23% $Fe^{2+}$ ion, respectively, and this increased to 74.55%, 82.12% and 90.33%, respectively at 100 µg/mL. $IC_{50}$ values indicated that the root alkaloid fraction was the most potent $Fe^{2+}$ chelator ($IC_{50}$ = 12.01 µg/mL) and the leaf alkaloid fraction the least ($IC_{50}$ = 17.12 µg/mL).

*Antibacterial activity*

The antibacterial activity of alkaloid fractions of *T. hirtum* against clinical isolates of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella typhi* was determined by the agar well diffusion method and MIC and MBC evaluated by the broth microdilution method. In the agar well diffusion method, results revealed that the alkaloid fractions were potent inhibitors of bacterial growth in a dose-dependent manner (Table 2).

At 1 mg/mL, the stem alkaloid fraction inhibited the growth of all tested microorganisms except *E. coli*. Similar result was obtained for the root alkaloid fraction, which was active against the tested isolates except *E. coli* and *S. typhi* at the same concentration. The leaf alkaloid fraction inhibited the growth of all the tested bacterial isolates at the lowest concentration of 1 mg/mL.

At 3 mg/mL, the leaf alkaloid fraction showed prominent inhibition against *S. aureus and E. coli* with inhibition zone diameters of 16.40 mm and 13.10 mm, respectively, while the stem alkaloid fraction was most effective against *B. subtilis* and *P. aeruginosa* with inhibition zone diameters of 18.70 mm and 12.70 mm at the same concentration. Similarly, the root alkaloid fraction exhibited considerable inhibition of bacterial growth against *S. typhi* at this concentration. Lower inhibition zones were reported for alkaloid fractions of *P. juliflora* against *E. coli* and *S. aureus*[17] and alkaloid extracts of *A. articulata* against *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa.*[28] However, these were inferior to the standard drug ciprofloxacin.

MIC and MBC were determined by the broth microdilution technique as presented in Table 3. The alkaloid fractions exhibited remarkable antibacterial activity against the test organisms with MIC values that ranged from 0.09 to 2.28 mg/mL. MBC were two or three times higher than the MIC and these differences were significant (p = 0.05). With regards to the leaf alkaloid fraction, the highest antibacterial activity was demonstrated against *S. aureus* (MIC = 0.09 mg/mL) followed by *E. coli* (MIC = 0.30 mg/mL), while the least activity was against *S. typhi* (MIC = 2.28 mg/mL). The stem alkaloid fraction demonstrated significant antibacterial activity against *S. aureus, B. subtilis* and *P. aeruginosa* with MIC values of 0.17, 0.24 and 0.46 mg/mL, respectively, while the least antibacterial activity was exerted against *S. typhi* (MIC = 1.67 mg/mL). Again, for the root alkaloid fractions, promising antibacterial activity was observed against *B. subtilis*, *S. aureus* and *S. typhi* (MIC = 0.33, 0.42 and 0.53 mg/mL, respectively), suggesting that the alkaloid fractions exhibited a broad-spectrum antibacterial activity. The response of the organisms however, varied with exposure to the extracts. For instance, *S. aureus* was sensitive to all the alkaloid fractions; *B. subtilis* to stem and root alkaloid fractions, while the other microorganisms were sensitive to either the leaf, stem or root alkaloid fractions.

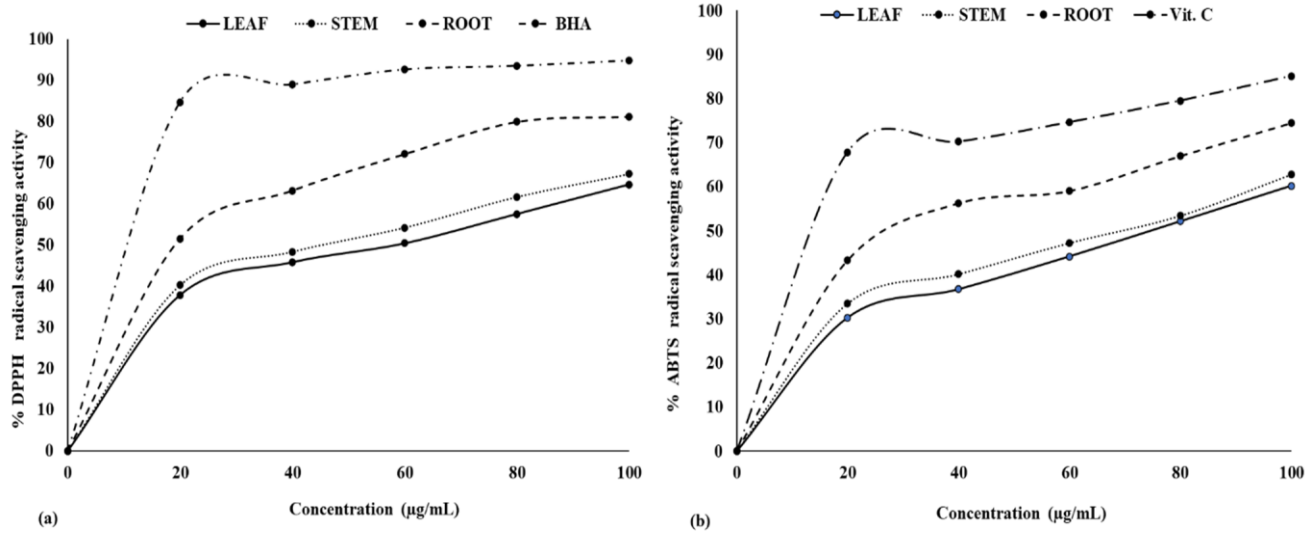


**Figure 1: (a)** DPPH radical scavenging activity of *T. hirtum* alkaloid fractions, **(b)** ABTS radical scavenging activity of *T. hirtum* alkaloid fractions.

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**Table 1:** Antioxidant activity of alkaloid fractions of *T. hirtum* root.

| Parameter | Fractions | | | Controls | | |
|---|---|---|---|---|---|---|
| | Leaf | Stem | Root | BHA | EDTA | Vit. C |
| DPPH scavenging activity * | 58.83 | 44.35 | 19.34 | 10.07 | - | - |
| ABTS scavenging activity * | 75.48 | 68.04 | 26.02 | - | - | 13.50 |
| $H_2O_2$ scavenging activity * | 78.86 | 17.46 | 20.02 | - | - | 13.78 |
| Reducing power * | 20.04 | 24.00 | 38.41 | - | - | 9.88 |
| Metal chelating activity * | 17.12 | 13.84 | 12.01 | - | 9.04 | - |

*$IC_{50}$ (µg/mL) is the effective concentration where ABTS, DPPH and $H_2O_2$ radical is scavenged by 50%, ferrous ion is chelated by 50% and the absorbance is 0.5 for reducing power. $IC_{50}$ was obtained using the regression equation.

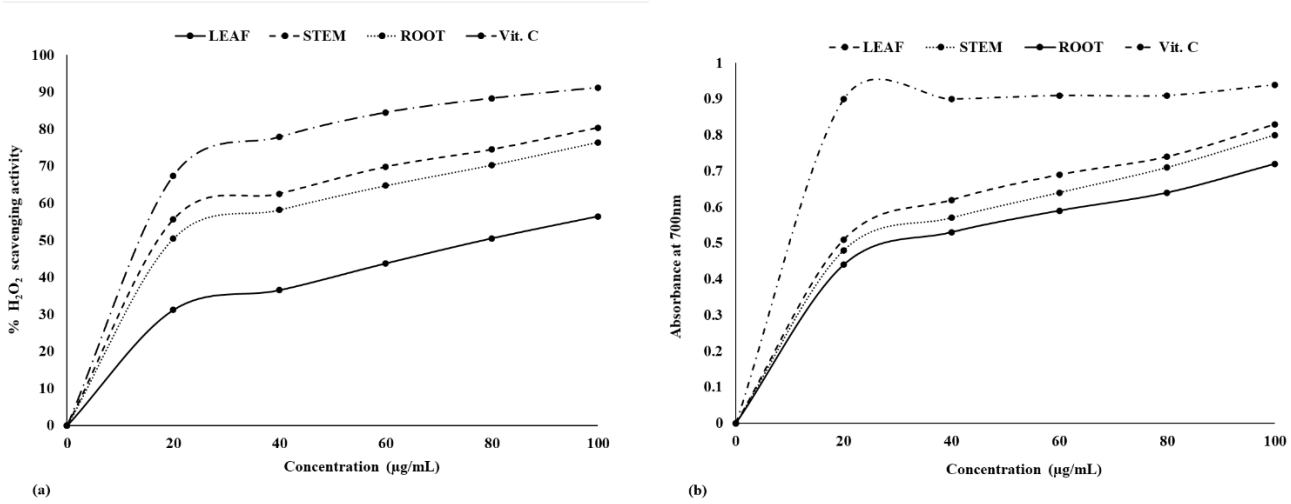


**Figure 2: (a)** $H_2O_2$ scavenging activity of *T. hirtum* alkaloid fractions, **(b)** Reducing power of *T. hirtum* alkaloid fractions.

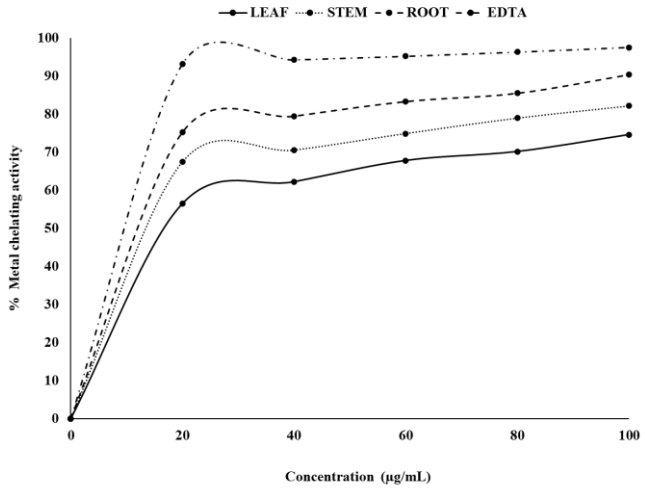


**Figure 3:** Metal chelating activity of *T. hirtum* alkaloid fractions.

The evidence suggests that Gram-positive organisms, *S. aureus* and *B. subtilis* were the most susceptible species encountered in this study. This is in agreement with reports that Gram-positive microorganisms are usually more sensitive to extract or fractions than Gram-negative microorganisms due to their morphological variation.[29] Lower MIC value of 0.0025 mg/mL for alkaloid extract of C. *citrinus* against *S. aureus*, but similar MIC values (MIC = 0.21-0.41 mg/mL) for alkaloid extract of C. *citrinus* and *V. adoensis* against *P. aeruginosa* have been reported.[1] In addition, Kucukboyaci *et al.*[30] reported lower MIC values of 65.5 to 500 µg/mL for alkaloid extracts of *S. alopecuroides* against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa*,[30] and MIC values of 62.5, 62.5, 500 and 62.5 µg/mL for alkaloid extract of *L. angustifolius* against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa*, respectively.[31] Generally, the results in this study indicate that alkaloid fractions from organs of *T. hirtum* exerted potent antibacterial activity against both Gram-positive and Gram-negative bacteria, with the leaf, stem and root alkaloid fractions being very active against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* (MIC < 0.5 mg/mL). This is in agreement with numerous reports that alkaloid extracts possess antimicrobial properties.[32] Possible mechanisms of action for the antimicrobial activity of alkaloids include inhibition of cell division, respiratory and enzyme inhibition, bacterial membrane disruption and suppression of virulence genes.[32] From these we can infer that the observed activity of the *T. hirtum* alkaloid fractions and bacterial response occurred because of one or a combination of these mechanisms.

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**Table 2:** Inhibition zone diameters of alkaloid fractions of *T. hirtum* against test organisms

| Alkaloid fraction / Control | | Inhibition zone diameters (mm)* | | | | |
|---|---|---|---|---|---|---|
| | | Gram positive organisms | | Gram negative organisms | | |
| | | *S. aureus* | *B. subtilis* | *E. coli* | *P. aeruginosa* | *S. typhi* |
| Leaf | 1 mg/mL | 9.40 | 6.60 | 10.00 | 4.10 | 4.80 |
| | 2 mg/mL | 11.30 | 8.20 | 12.20 | 5.80 | 5.30 |
| | 3 mg/mL | 16.40 | 10.00 | 13.10 | 8.20 | 7.90 |
| Stem | 1 mg/mL | 7.30 | 9.30 | - | 8.00 | 6.00 |
| | 2 mg/mL | 9.70 | 13.60 | 6.80 | 10.20 | 7.80 |
| | 3 mg/mL | 13.20 | 18.70 | 10.50 | 12.70 | 9.30 |
| Root | 1 mg/mL | 5.00 | 10.30 | - | 7.90 | - |
| | 2 mg/mL | 7.80 | 14.30 | 6.00 | 9.00 | 11.20 |
| | 3 mg/mL | 9.50 | 17.20 | 8.30 | 10.50 | 12.80 |
| Ciprofloxacin | 20 µg/mL | 27.40 | 24.50 | 28.40 | 30.30 | 29.00 |

* mean of triplicate determinations.

**Table 3:** MIC and MFC of alkaloid fractions of *T. hirtum* against test organisms*

| | | Alkaloid fractions | | | | | | Control | |
|---|---|---|---|---|---|---|---|---|---|
| | | Leaf | | Stem | | Root | | Ciprofloxacin | |
| **Bacteria** | | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** |
| Gram (+ve) | *S. aureus* | 0.09 | 0.18 | 0.17 | 0.51 | 0.42 | 0.84 | 0.06 | 0.06 |
| | *B. subtilis* | 0.85 | 1.70 | 0.24 | 0.48 | 0.33 | 0.66 | 0.02 | 0.04 |
| Gram (-ve) | *E. coli* | 0.30 | 0.60 | 1.02 | 2.04 | 2.13 | 4.26 | 0.03 | 0.03 |
| | *P. aeruginosa* | 1.81 | 3.62 | 0.46 | 0.92 | 1.08 | 2.16 | 0.03 | 0.06 |
| | *S. typhi* | 2.28 | 4.56 | 1.67 | 3.34 | 0.53 | 1.06 | 0.04 | 0.04 |

* mean of triplicate determinations, concentration of alkaloid fractions in mg/mL and ciprofloxacin in µg/mL.

## Conclusion

Alkaloid fractions from organs of *T. hirtum* exhibited potent radical scavenging, metal chelating and reducing activities in the tested models. Furthermore, the fractions demonstrated promising antibacterial activity against the test organisms. These results suggest that alkaloid fractions from organs of *T. hirtum* are effective natural and alternative sources of compounds with antioxidant and antibacterial properties.

## 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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