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# Pharmacological Insights into *Trichodesma indicum*: A Medicinally Valuable Herb

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**ABSTRACT**

**Background:** The present study was aimed to investigate the pharmacological properties of a medicinally significant herbal species, *Trichodesma indicum*. Phytochemical analysis of methanol, ethanol, acetone, petroleum ether and aqueous extracts of *T.indicum* was explored.

**Methodology:** Antibacterial activity of these solvent extracts of *T.indicum* was determined against the test organisms (*Escherichia coli, Klebsiella* sp., *Pseudomonas aeruginosa, Salmonella* sp. and *Proteus* sp.) using well diffusion method. Anti-oxidant activity for three different standard concentrations (100, 200 and 300µg/ml) of *T.indicum* methanol extract was determined using DPPH, superoxide scavenging activity and nitric oxide scavenging activity assays. Wound healing efficacy of *T.indicum* methanol extract was investigated using standard *in vitro* wound scratch assay.

**Results:** Phytochemical analysis explored the presence of alkaloids, phenols, flavonoids, tannins and terpenoids in higher level for methanol extracts of *T.indicum.* Antibacterial activity of the methanol extracts of *T.indicum* exhibited inhibitory zones ranging from 27 to 29mm against all the test bacteria; which was comparatively maximum than other solvent extracts. Hence methanol extract was used for anti-oxidant activity and wound healing studies. Anti-oxidant DPPH assays revealed IC50 value of 292.19µg/ml for *T. indicum* extract. Superoxide scavenging activity expressed IC50 value of 192.34µg/ml; and nitric oxide scavenging activity showed IC50 value of 224.64µg/ml respectively. The self-wound healing ability of *T.indicum* extracts showed positive cell migration and cell proliferation after 12 $^{th}$ and 24$^{th}$ hours indicating the wound closure under *in vitro* conditions.

**Conclusion & recommendation:** The research showed promising results and evidenced pharmacological properties of the selected medicinally significant *T.indicum.* Further studies like anti-diabetic, antifungal and antiviral properties shall be considered based on the above results.

**KEYWORDS:** Soxhlet extraction, Anti-oxidant activity, Wound healing, Scratch assay, Phytochemicals.

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## I. INTRODUCTION

The World Health Organization (WHO) recommends swift action to mitigate the rapidly growing threats of antimicrobial resistance, and there is an urgent need to discover new antimicrobial agents from natural sources (Viswapriya *et al.,* 2022). Searching for new antimicrobial agents is a demanding one where the irrational use of antimicrobials develops drug resistance, causing potential biohazards (Wasihun *et al.,* 2023). Plants were used in simple or complex forms, i.e., crude extracts, mixtures, etc., which accounts for the number of new drugs developed and used against many diseases (Vaou *et al.,* 2021). Medicinal plants are the major source of treatment and preventive agents with a long history of applications (Najmi *et al.,* 2022).

Many literature surveys showed that plant- based drugs play a promising role in the treatment of infectious diseases. Bioactive components of plants include array of compounds (e.g., tannins, lignans, coumarins, quinones, stilbenes, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins, and proanthocyanins) that could

delay or inhibit the inception of degenerative diseases (Adebo *et al.,*2020) and increase life expectancy. Infectious diseases are also the major cause of mortality worldwide. The bioactive compounds in plants lead to their fundamental role in modern drug development (Ugboko *et al.,* 2020). Owing to the medicinal usage of the study plants, the present study was made to evaluate the antimicrobial properties of *T.indicum* leaf extracts towards clinical isolates. Leaf extracts of *T.indicum* were reported to be effective in treating inflammations in the joints (Tresina *et al.,* 2023) and wound healing (Rangasamy *et al.,* 2023).

Many researches were carried throughout India about the pharmacognosy, phytochemistry, ethanopharmacology and pharmacological properties of *T.indicum.* Each part of the plant was reported for its antioxidant, anti-inflammatory, analgesic, antipyretic, antimicrobial and anti-diabetic activity. Hamsalakshmi and her co-workers reported that *Trichodesma indicum* commonly known as Adhapushpi and belong to the family Boraginaceae. They reviewed that, the extracts was used for different medicinal applications like arthritis, fever, skin disease, arthralgia and dysentery (Hamsalakshmi *et al.,* 2018). Sardar *et al.,* investigated the ethanobotanical novelty and usefulness of *T.indicum* among the people of Paliyar tribal village, Tamil Nadu. The researchers reported that *T.indicum* leaf extracts was found to be useful in the treatment of ear pains and wound healing (Sardar *et al.,* 2022). Mazhar *et al.,* studied the phytochemical, anti-oxidant and antimicrobial activities of *T.indicum* using different solvent extracts (Mazhar *et al.,* 2022).

The researchers found different phytochemical compounds like tannins, steroids and terpenoids. Solvent extracts also showed promising antibacterial and antifungal activity with good anti-oxidant scavenging activities. Based on these significant medicinal values of *T.indicum*, following objectives were framed in the present study. To investigate the phytochemical analysis for the different solvent extracts of *T.indicum* and to determine the antibacterial activity of *T.indicum* solvent extracts. To evaluate the anti-oxidant activity and wound healing ability of *T.indicum* extract. All the objectives were carried out using standard experimental protocols and the results were found supportive for the framed objectives.

## II. MATERIALS AND METHODS

### 2.1 Selection of Herbs

*T.indicum* leaves were selected in the present study based on its medicinal properties as this plant was used by Malasar tribes, Coimbatore, Tamil Nadu, India.

### 2.2 Procurement and extraction of *T.indicum* (Joghee *et al.,* 2021)

*T.indicum* leaves were collected from the farmhouse, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The plant leaves were collected from in and around Coimbatore region and were authenticated as *T.indicum* at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Using different solvents (methanol, ethanol, acetone, petroleum ether and water) the extractions of the leaf powders were carried out using a standard Soxhlet extraction apparatus. Collected extracts were transferred to petridish separately and dried in oven at 50°C and the extracts were scrapped and kept in air tight brown amber containers at room temperature.

### 2.3 Phytochemical analysis

Phytochemical analysis of different solvent extracts of *T.indicum* was subjected to detect the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides, phenols, carbohydrates and proteins using standard method (Abdelgawad *et al.,* 2021).

### 2.4 Antibacterial activity of *T.indicum* extracts

Antibacterial activity of different solvent extracts of *T.indicum* was determined against the test organisms (*E.coli*, *Klebsiella* sp., *P.aeruginosa, Salmonella* sp. and *Proteus* sp.) using well diffusion method. A sterile Nutrient broth (g/L) Peptone: 5g, Yeast extract: 5g, Beef extract: 3g, Sodium chloride: 5g, Final pH - 7.0 ± 0.2) was used to inoculate all of the test cultures, and they were then given 24 to 48 hours to proliferate. Plates of sterile Mueller-Hinton Agar (MHA) were made and allowed to set up. Swabs were used to evenly spread 0.1% inoculum suspensions of the test organism over the agar surface in each case. On the agar surface of each plate, 6mm wells were cut while maintaining sterility. About 20µl of each solvent extracts of *T.indicum* (at concentration of 100µg/ml) was added to each well. The plates were then incubated at 37°C for 24 hours to observe significant inhibitory zones after incubation period.

### 2.5 Antioxidant activity

#### 2.5.1 DPPH

Three different standard concentrations (100, 200 and 300µg/ml) of *T.indicum* methanol extract was prepared using DMSO. Each concentration of samples was mixed with equal volume of DPPH (0.3mM). The mixture was allowed to react at room temperature in the dark for 30 minutes. Ascorbic acid was used as standard controls. Three replicates were made for each test sample. After 30 minutes, the absorbance (A) was measured at 518nm and converted into the percentage antioxidant activity using the following equation:

$I\ (\%) = 100\times (A_0-A_1)/A_o$

Where $A_0$ is the absorbance of the control, A1 was the absorbance in the presence of the sample of extract respectively.

#### 2.5.2 Superoxide scavenging activity

Superoxide scavenging activity of *T.indicum* methanol extract was measured by the reduction of nitroblue tetrazolium (NBT) according to a previously reported method (Chhajed *et al.,* 2023). The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which

reduce nitro blue tetrazolium (NBT) to a purple formazan. Quercetin was used as positive control. The percentage inhibition of super-oxide anion generation was calculated using,

Superoxide anion radical scavenging (%) = [(Ac – As)] × 100

Where Ac is the absorbance of the control reaction (superoxide radical + methanol) and As is the absorbance of the test samples (extract or standard).

**2.5.3 Nitric oxide scavenging activity (Van Vuuren *et al.*, 2020)**

Nitric oxide scavenging activity of *T.indicum* methanol extract was determined by the method suggested by Van Vuuren and his co-workers. Briefly, 0.5ml of the extract or standard (ascorbic acid) at different concentrations (100-300µg/ml) was mixed with sodium nitro-prusside (2ml, 10mM) prepared in 0.5mM phosphate buffer saline (pH 7.4). About 0.5ml of mixture was mixed with 0.5ml of Griess reagent and the absorbance was measured at 540nm.

Nitric oxide radical scavenging activity (%) = [(Ac - As)/Ac] × 100

Where Ac is the absorbance of the control (Nitric oxide radical + methanol) and As is the absorbance of the test samples (extract or standard).

**2.5.4 Analyzing the wound healing ability: in vitro wound scratch assay (Fathil *et al.*, 2023)**

The wound healing efficacy of the plant extract was determined based on the migration rates of fibroblast cells using scratch assay method. The cell density of 2 × 105 cells were seeded into each well of a 24-well microtiter plate and incubated with a complete medium at 37 °C and 5% CO2. The monolayer confluent cells were scrapped horizontally with a sterile P200 pipette tip after 24 hours of incubation and the debris were removed by washing with PBS. The cells were treated with *T. indicum* extracts (100µg/ml) by diluting with serum-free Dulbecco Modified Eagle Medium (DMEM). The cells treated with allantoin (Sigma Aldrich, Germany) were used as the positive control. The scratch, induced as a wound, was photographed at the $0^{th}$ hour using phase contrast microscopy at 40X magnification. Wound closure was determined based on the migration rates of fibroblast cells after $12^{th}$ and $24^{th}$ hour, and the results were compared with the control.

## III. RESULTS AND DISCUSSION

### 3.1. Phytochemical analysis

*T.indicum* was used in the ancient days for treating wounds, ulcers, chronic fever, rheumatism, dysentery, and anemia. Their phytochemical constituents play a vital role in the field of pharmacological science as antioxidant, anticancer, and antibacterial compounds. In the present study, phytochemical analysis of the plant extracts revealed the presence of different bioactive compounds. In Table 1, the presence and absence of the compounds were expressed in terms of positive and negative signs.

**Table- 1 Phytochemical analysis of *T. indicum***

| S. No | Phytochemicals | *T. indicum* extracts | | | | |
|---|---|---|---|---|---|---|
| | | Methanol | Ethanol | Acetone | Petroleum ether | Water |
| 1 | Alkaloids | ++ | ++ | + | + | + |
| 2 | Flavonoids | + | + | + | - | - |
| 3 | Terpenoids | ++ | ++ | + | + | - |
| 4 | Phenols | ++ | + | + | + | + |
| 5 | Tannins | ++ | + | - | + | - |

**Keywords:** +Present, -Absent

From the obtained results, it was evident that alkaloids, phenols, flavonoids, tannins, and terpenoids were found to be present at a higher level in the methanol extracts of *T.indicum* (Fig. 1). Ndezo Bisso *et al.*, in his studies demonstrated that flavonoids, phenols, tannins, terpenoids, and steroids were found in the ethyl acetate extract (Ndezo Bisso *et al.*, 2022).

**Fig. 1: Phytochemical analysis of *Trichodesma indicum* (Methanol extract)**

Acetone and petroleum ether extracts showed the presence of alkaloids, phenols, flavonoids, and terpenoids at comparatively lower levels than methanol extracts. Water extracts showed the presence of only phenols and tannins. The presence of many significant phytochemical compounds was found to be attributable to different biological properties like antibacterial, antifungal, antioxidant, and anticancer. Based on these factors, antibacterial activity and antioxidant activity were determined in our study.

**3.2 Antibacterial activity of *T. indicum* among selected solvents**

The antibacterial activity of different solvent and aqueous extracts of *T. indicum* was presented in Table 2, and the inhibitory zones produced against the respective test bacteria were presented in Fig. 2.

**Table-2: Antibacterial activity of different solvent extracts of *Trichodesma indicum***

| S. No | Test Bacteria | Zone of Inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Methanol | Ethanol | Acetone | Petroleum ether | Water | Positive control | Negative control |
| 1 | *E. coli* | 27 | 21 | 20 | 25 | 17 | 29 | 0 |
| 2 | *Klebsiella* sp. | 28 | 24 | 22 | 21 | 0 | 30 | 0 |
| 3 | *P. aeruginosa* | 27 | 22 | 20 | 20 | 0 | 28 | 0 |
| 4 | *Salmonella* sp. | 28 | 23 | 21 | 20 | 16 | 31 | 0 |
| 5 | *Proteus* sp. | 28 | 24 | 21 | 20 | 17 | 30 | 0 |

**Fig. 2: Antibacterial activity of different solvent extracts of *Trichodesma indicum***

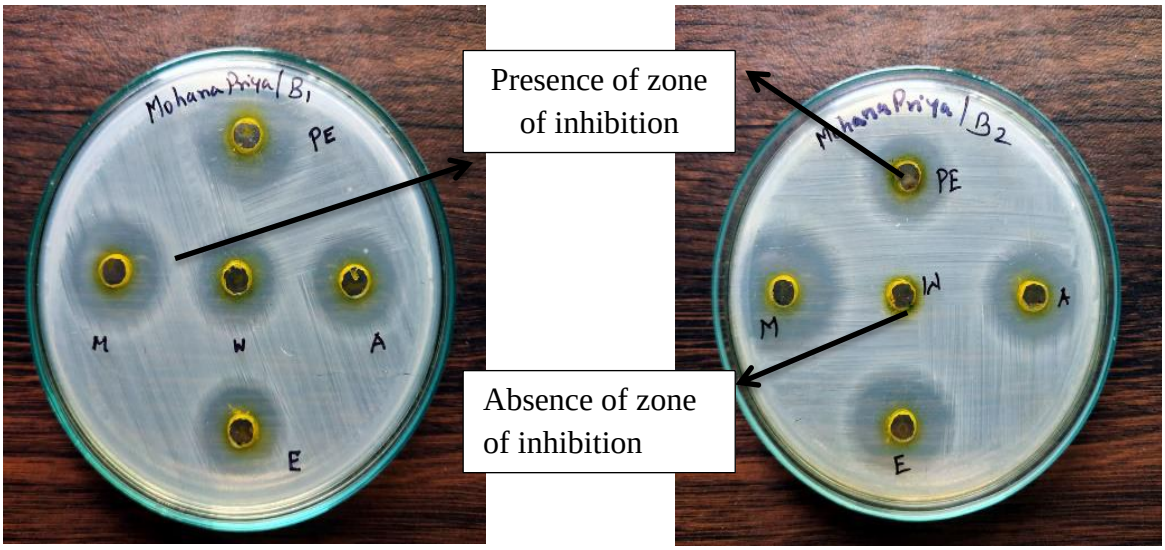



**Fig. 2** Plates showing absence and presence of clear halo zones (a) and (b)

a) *E. coli* b) *Klebsiella* sp

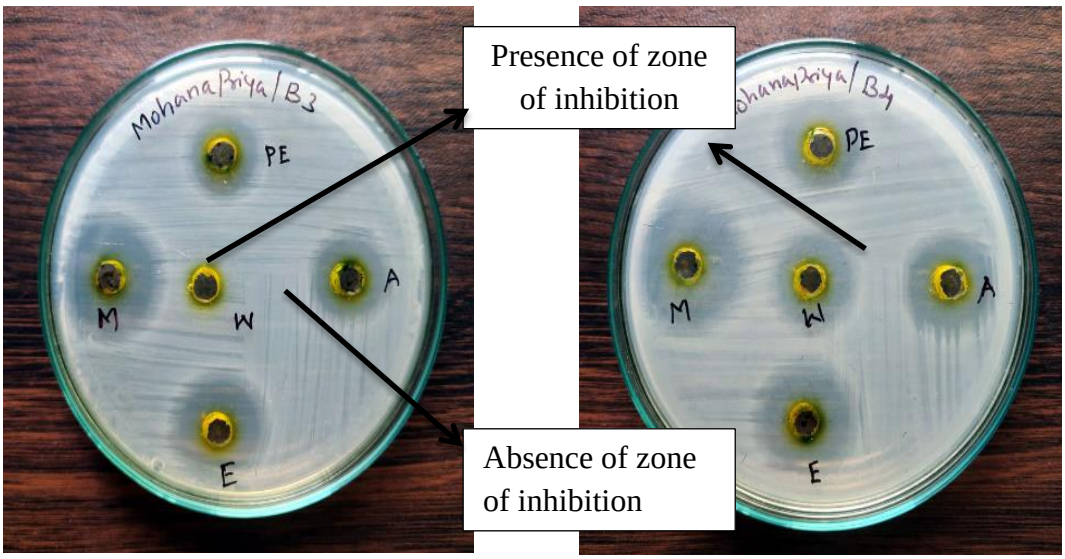



**Fig. 2** Plates showing absence and presence of clear halo zones (c) and (d)
c) *P. aeruginosa* d) *Salmonella* sp

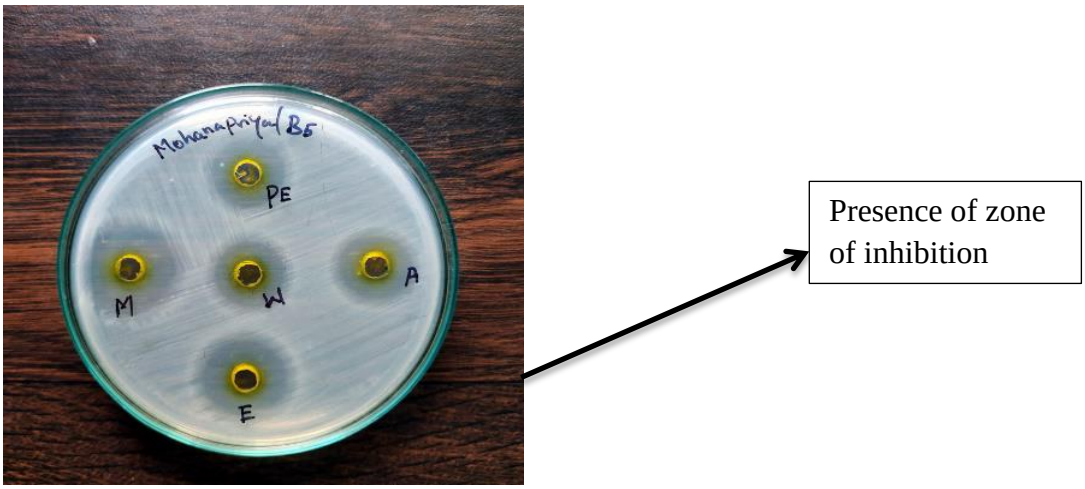



**Fig. 2** Plate showing presence of clear halo zones (e) *Proteus* sp

**3.2.1 Antibacterial activity of methanol extract**

The maximum inhibitory zone against Salmonella sp. was around 29 mm, while *Klebsiella* sp. and *Proteus* sp. shared an inhibitory zone of approximately 28 mm. The least zone of inhibition, recorded at 27 mm, inhibited *P.aeruginosa* and *E .coli*.

**3.2.2 Antibacterial activity of ethanol extract**

A maximum inhibitory zone around 24 mm was reported against *Klebsiella* sp. and *Proteus* sp.; subsequently, 23 mm was recorded against Salmonella sp. *P.aeruginosa* and *E. coli* were inhibited with zones measuring 22 and 21 mm, respectively.

**3.2.3 Antibacterial activity of acetone extract**

The maximum inhibitory zone corresponded to 22 mm against *Klebsiella* sp, while it was close to 21 mm against the other two test organisms, *Salmonella* sp. and *Proteus* sp. *P.aeruginosa* and *E.coli* were inhibited at about 20 mm each.

**3.2.4 Antibacterial activity of petroleum ether extract**

A maximum inhibitory zone of about 25 mm was observed against *E.coli*. About 20 mm of inhibitory zones were exhibited against *P.aeruginosa*, *Salmonella* sp., and *Proteus* sp., respectively, and a zone of clearance of about 21 mm was noted against *Klebsiella* sp.

**3.2.5 Antibacterial activity of water extract**

A maximum inhibitory zone measuring around 17 mm was recorded against *E.coli* and *Proteus* sp. A relatively close margin of around 16 mm was reported against *Salmonella* sp. absolutely no zones were recorded against *P.aeruginosa* and *Klebsiella* sp. Based on the results of the phytochemical analysis and antibacterial activity, the methanol extracts of *T. indicum* were found to be very effective, and so they were employed to carry out anti-oxidant and wound healing investigations.

The antibacterial activity of *T.indicum* expressed in the present study was generally reasoned in many research articles to support the results of our present study. The

antimicrobial properties of secondary metabolites in plant extracts and their mode of action were reported to be dependent on several factors (Allemailem *et al.*, 2021; Anand *et al.*, 2020).

The antibacterial action generally includes the following sequence of events: Plant phytochemicals interact with the cell membrane, diffuse through the membrane (phytochemicals from the plant penetrate into the interior of the cell), and interact with intracellular constituents (Haq *et al.*, 2019). Different phytochemical compounds, especially terpenoids, triterpenoids, and phenols from plant extracts, were found to be responsible for the above-mentioned mode of action on the cell membrane of bacteria. Ali and his co-workers found that terpenoids and triterpenoids were involved in the antibacterial activity of *T.indicum* (Ali *et al.*, 2022). Similarly, the study revealed terpenoids found in the methanol and ethanol extracts of *T. indicum* expressed potent antibacterial activity against all the test bacteria.

### 3.3 Antioxidant activity

#### 3.3.1. DPPH

Antioxidants have been recognized to exhibit protective functions against oxidative damage and are associated with reduced risk of chronic diseases (Balan *et al.*, 2015). In this study, the antioxidant activities of the *T. indicum* methanolic leaf extract was evaluated using DPPH scavenging ability.

**Fig. 3: DPPH radical scavenging activity of *Trichodesma indicum* extract**

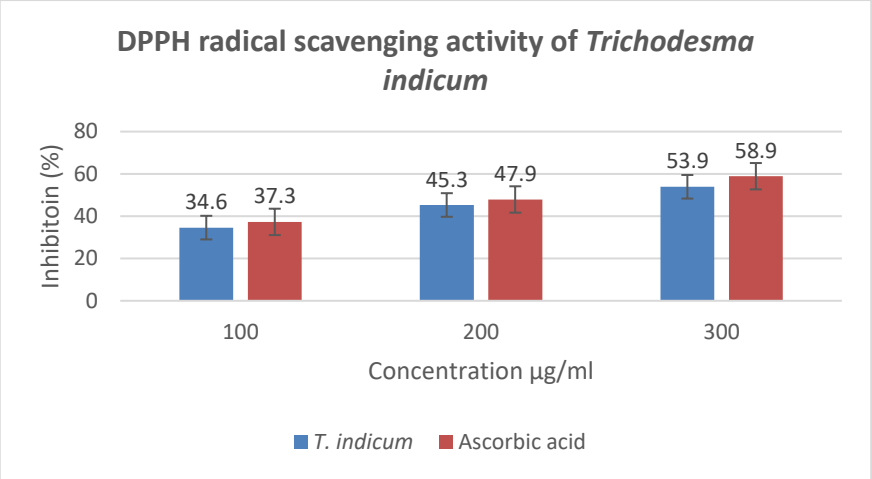



Fig. 3 Shows free radical scavenging activity of *T. indicum* and standard ascorbic acid at concentration of 100–300µg/ml. Ascorbic acid was taken as a positive control (standard). The IC50 values were found to be 292.19µg/ml and 231.16µg/ml for *T. indicum* and ascorbic acid, respectively (Table-3).

**Table-3: DPPH radical scavenging activity of *Trichodesma indicum* extract**

| S. No. | Concentration (µg/ml) | Inhibition (%) | |
|---|---|---|---|
| | | Herbal extract | Ascorbic acid |
| 1 | 100 | 34.6 ± 0.75 | 37.3 ± 1.05 |
| 2 | 200 | 45.3 ± 1.05 | 47.9 ± 0.57 |
| 3 | 300 | 53.9 ± 0.57 | 58.9 ± 0.57 |
| $IC_{50}$ Value (µg/ml) | | 292.19µg/ml | 231.16µg/ml |

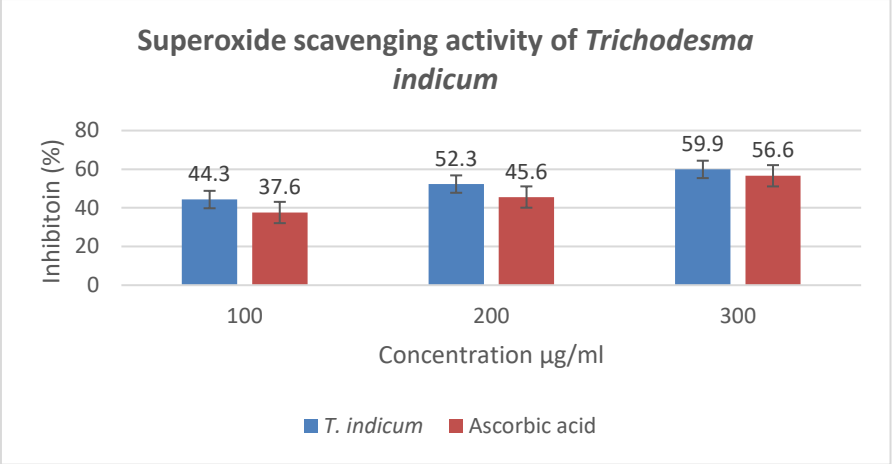
The results showed that antioxidant activity of *T. indicum* in dose dependent manner. In this experiment, the role of stable free radical of DPPH is to react with antioxidative free radicals of methanol leaf extract of *T. indicum*. The violet color of stable free radical is converting to pale color with the progress in reaction of antioxidant free radicals of the *T. indicum* extract. The rate of decolouration of *T. indicum* represents the strength of antioxidant activity. Phenolic and aromatic compounds can act as antioxidants by donating hydrogen to free radical and become themselves a radical, which will be stabilized by the resonance delocalization of the electron within the aromatic ring and formation of quinone structures.

Then, free radical scavenging ability increases as the number of phenolic hydroxyl groups increases. The reaction of *T. indicum* methanol leaf extract with a deep violet stable radical DPPH is based on the conversion of it into the decolourised radical α, α–diphenyl-β- picrylhydrazine due to their antioxidant property. In another report, it was showed that small molecules can access the DPPH radical better than larger ones and appeared to change color more quickly. This might have played a significant role in eradicating the radical (Shinde *et al.*, 2022).

**3.3.2. Superoxide scavenging activity**

The superoxide anions damage biomolecules directly or indirectly by forming H2O2, ¯OH, per oxy nitrite or singlet oxygen during aging and pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation (Laila *et al.*, 2023). The superoxide radical scavenging activities of methanolic leaf extract of *T. indicum* and ascorbic acid were widely varied. Increasing the sample concentration range from 100-300µg/ml, the scavenging effect also increased in the dose dependent manner (Fig. 4).

**Fig. 4: Superoxide scavenging activity of *Trichodesma indicum* extract**




The IC50 of *T. indicum* and ascorbic acid was 192.34 and 242.65µg/ml respectively (Table-4).

**Table-4: Superoxide scavenging activity of *Trichodesma indicum* extract**

| S. No. | Concentration (µg/ml) | Inhibition (%) | |
|---|---|---|---|
| | | Herbal extract | Ascorbic acid |
| 1 | 100 | 44.3 ± 1.05 | 37.6 ± 0.75 |
| 2 | 200 | 52.3 ± 1.05 | 45.9 ± 0.57 |
| 3 | 300 | 59.9 ± 0.57 | 56.6 ± 0.75 |
| $IC_{50}$ Value (µg/ml) | | 192.34µg/ml | 242.65µg/ml |

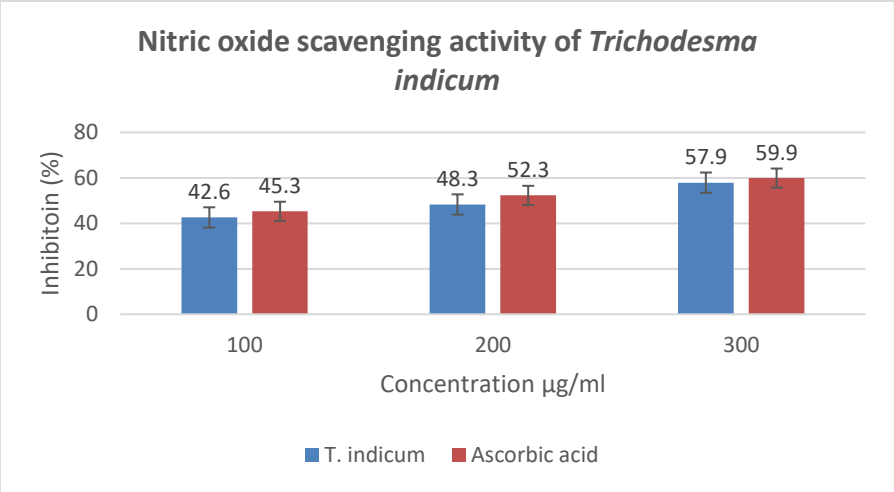
The secondary metabolites are effective antioxidants mainly because they scavenge superoxide anions. Superoxide anions are a precursor to active free radicals that have potential of reacting with biological macromolecules and thereby inducing tissue damage (Gulcin *et al.,* 2020). It has been implicated in several pathophysiological processes is due to its transformation into more reactive species such as hydroxyl radical that initiate lipid peroxidation. Also, superoxide has been observed to directly initiate lipid peroxidation (Hasan *et al.,* 2023). In addition, it has been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical (Aydın Kurç *et al.*, 2023).

Superoxide radical is normally formed first, and its effects can be magnified because it produces other kinds of free radicals and oxidizing agents (Wojtasińska *et al.*, 2023). Superoxide anion is derived from dissolved oxygen by riboflavin/methionine/illuminate system and reduces NBT in this system. In this method, superoxide anion reduces the yellow dye ($NBT^{2+}$) to produce the blue formazan which is measured using spectrophotometer at 560nm. Antioxidants are able to inhibit the blue NBT formation (Mróz *et al.*, 2023; El Karkouri *et al.*, 2022).

**3.3.3. Nitric oxide scavenging activity**

Nitric oxide (NO) or reactive nitrogen species such as $NO_2$ and $NO_3$ are formed during the reactions of nitrogen with oxygen or with superoxide which are very reactive. These compounds are responsible for altering the structural and functional behavior of many cellular components. Plant/plants products may have the property to counteract the effect of nitric oxide formation and in turn may be of considerable interest in preventing ill effects of excessive nitric oxide generation in the human body. Nitric oxide is also implicated for inflammation, cancer, and other pathological conditions (Andrabi *et al.*, 2023). In this study, the antioxidant activities of the *T. indicum* methanolic leaf extract was evaluated using nitric oxide radical scavenging ability (Fig. 5).

**Fig. 5: Nitric oxide scavenging activity of *Trichodesma indicum* extract**




Ascorbic acid (standard) used as a positive control. The IC50 (concentration that can scavenge 50% of the radical) values of *T. indicum* and ascorbic acid were noted to be 224.64 and 180.52µg/ml respectively (Table-5).

**Table-5: Nitric oxide scavenging activity of *T. indicum* extract**

| S. No. | Concentration (µg/ml) | Inhibition (%) | |
|---|---|---|---|
| | | Herbal extract | Ascorbic acid |
| 1 | 100 | 42.6 ± 0.75 | 45.3 ± 1.05 |
| 2 | 200 | 48.3 ± 1.05 | 52.3 ± 1.05 |
| 3 | 300 | 57.9 ± 0.57 | 59.9 ± 0.57 |
| $IC_{50}$ Value (µg/ml) | | 224.64µg/ml | 180.52µg/ml |

Nitric oxide radical is produced from sodium nitro-prusside in aqueous solution, which reacts with oxygen to form nitrile. However, over production of this radical has been implicated in the pathogenesis of various diseases such as diabetes, carcinomas and arthritis. The results of nitric oxide radical scavenging activity showed that methanol extract of *T. indicum* inhibited the formation of nitric oxide. This may be due to the antioxidant attitude in the methanol leaf extract *T. indicum*, which competed with oxygen to react with nitric oxide and thus inhibited generation of nitrile radicals (Stavely *et al.*, 2023). In correlation with this report, the present study showed a strong nitric oxide radical scavenging activity of *T. indicum* methanol extract (Abifarin *et al.*, 2020). The activity of methanol leaf extracts of these plants may possibly help to stop the chain reaction instigated by excessive production of nitric oxide and may play a significant role in preventing inflammatory signaling processes involving nitric oxide (Sagbo *et al.*, 2017).

### 3.4. Wound healing activity

*In vitro* wound healing assays have commonly been applied to measure cell migration, cell proliferation, and wound closure in response to stimulation with specific agents. In this study, the AMPs-CuNPs conjugates used for the cell adhesion studies were determined for their ability to improve wound healing by acting directly on L929 mouse fibroblast cells. After creating a scratch on L929 mouse fibroblast cell lines, the cell migration, cell proliferation, and wound closure were measured for a known concentration (100µg) of *T. indicum* extracts at three different time periods ($0^{th}$ hour, $12^{th}$ hour, and $24^{th}$ hour). The self-wound healing ability of the plant extracts showed that, at $0^{th}$ hour, no cell migration and proliferation was observed for the known concentrate (100µg) including control. At the 12th hour, positive cell migration and cell proliferation were observed when compared to the control sample. After 24 hours, more cell proliferation was evident indicating the wound closure (Fig. 6).

**Fig. 6: *In vitro* wound scratch assay**


Fig. A: positive control without *T. indicum* extract

Fig. B: images showing the fibroblast migration induced by the sample containing 100µg of *T. indicum* extract

With support to the obtained results, very few researchers studied the wound healing activity of *Trichodesma indicum.* Sardar *et al*., (2022) reported that *T.indicum* leaf extracts was found to be useful in the treatment of wound healing. Abdelgawad and his collegues formulated white bees wax based cream containing 5% w/w of *Trichodesma indicum* solvent extracts (Abdelgawad *et al.,* 2021). Their report revealed that the herbal formulation of *Trichodesma indicum* extract has potential wound healing activity. The wound healing potential of *Trichodesma zeylanicum* extracts were evaluated after conducting a wound excision model in albino rats. The results showed good wound healing after specified incubation period when compared to positive and negative control animal groups (Thirumalai *et al.,* 2022).

Premkumar and his colleagues recently reported a case study on the efficacy of *T.indicum* cream on the local application of lacerated wound (Premkumar *et al.,* 2022). Varied results were found evident in the lacerated wound of the patient. Different observations in different patients were recorded like symptomatic relief in pain, skin colour of surrounding skin, discharge, tenderness and wound healing within 7 days.

## 4. CONCLUSION

*T. indicum* is regarded as a therapeutically essential medicinal plant species with a wide range of pharmacological properties, including antibacterial, antioxidant, and wound healing activities. Phytochemical study findings disclosed that methanol extracts of *T. indicum* included higher levels of alkaloids, phenols, flavonoids, tannins, and terpenoids. The methanol extracts of *T. indicum* have the highest inhibitory zones when compared to other solvent extracts. The anti-oxidant DPPH assay, superoxide scavenging activity, and nitric oxide scavenging activity exhibited IC50 values of 292.19µg/ml, 192.34µg/ml, and 224.64µg/ml, respectively.

In the in vitro wound scratch assay, *T. indicum* extracts demonstrated promising wound healing activity with efficient cell migration and proliferation after 12 and 24 hours. By considering the pharmacological capabilities of this plant species, we pave the way for further research into other pharmaceutical qualities such as anti-diabetic, antifungal, and antiviral properties.

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### Conflict of Interest

Authors declare no conflict of interest in the present study.

### Author's Contribution

First author contributed in performing all laboratory works. Second author provided all protocols to perform the laboratory experiments.

### Funding

No funds granted.

### Ethics Statement

No animals and humans used in the present research.

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