Comparative study to evaluate the anti-diabetic and anti-bacterial potential of *Tinospora cordifolia* and *Catharanthus roseus* plant extracts

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Abstract
Tinospora cordifolia (Giloy) and Catharanthus roseus (Sadabahar) possess an abundance of bioactive substances and other advantageous attributes. Methanolic extracts of the entire plant from both plants were examined in the in vitro investigation for their anti-bacterial and anti-diabetic properties. The results of the anti-bacterial activity demonstrated that the methanolic plants extract had anti-bacterial activity against Staphylococcus aureus, Escherichia coli and Bacillus subtilis bacteria.

In comparison to T. cordifolia, C. roseus showed the maximum zone of inhibition. Extracts from C. roseus exhibit a higher α-amylase inhibitory activity (55.43%) in comparison to extracts from T. cordifolia (53.44%) thus proving higher anti-diabetic potential. The methanolic extract of both plants contained phenols, flavonoids, proteins, carbohydrates and sterols, based on the results of the qualitative analysis of significant secondary metabolites. The current study has therefore concluded that T. cordifolia and C. roseus, two medicinal plants, may aid in the discovery of novel medications that may be used as treatments for bacterial and diabetes ailments that are resistant to current medications.

Keywords: Medicinal plants, Phytochemicals, Anti-bacterial, Anti-diabetic.

Introduction

Diabetes mellitus (DM) is a chronic illness that is highly prevalent globally[5]. One major global health concern is diabetes mellitus (DM) that impacts 537 million individuals worldwide. The WHO states that approximately 643 million people will have diabetes by 2030 and 783 million people will have the disease by 2045 due to the population's constant rise in diabetes sufferers[15].

Diabetic mellitus (DM) arises from either a genetic or acquired insufficiency in pancreatic insulin production, or from the ineffectiveness of insulin. Diabetic retinopathy, diabetic foot, diabetic neuropathy, diabetic nephropathy, atherosclerosis, diabetic retinopathy, diabetic nephropathy and cardiovascular disease are all consequences of diabetes mellitus (DM), a chronic endocrine disorder that causes hyperglycemia and severe irreversible microvascular and macrovascular complications[20].

The aforementioned issues might arise due to deficiencies in either insulin action (such as insulin resistance and hyperinsulinemia) or insulin secretion (i.e. inadequate or insufficient synthesis of insulin from the pancreas), or both. Variations in people's lifestyles, behaviors and environments all contribute to the rising rate of diabetes mellitus. The administration of insulin and various antidiabetic drugs, including sulfonylureas[11], metformin[7], glinides, biguanides and acarbose[9], are part of the standard treatment for diabetes mellitus. The majority of antidiabetic medications have negative consequences including weight gain, anemia, renal failure, gastrointestinal problems and hypoglycemia, despite the fact that they are effective in decreasing and stabilizing blood glucose levels.

Thus, the development of novel antidiabetic pharmaceuticals requires a focus on the quest for new natural remedies with safer and more effective qualities[14]. Medicinal plants are a great natural therapy substitute for synthetic medications because of their low toxicity, inherent antioxidants and significant bioactive phytochemicals[11]. It has been demonstrated that more than 400 herbal plants contain antidiabetic properties, indicating their importance in the therapy and control of diabetes[13].

It has been demonstrated that the antihyperglycemic action is brought by phyto active compounds found within herbal plants including alkaloids, polyphenols, terpenoids, flavonoids, saponins and tannins[17].

Since microbial infections are on the rise, they have emerged as a serious clinical concern with substantial associated morbidity and mortality[3]. As a result, methods for analyzing antimicrobial susceptibility and finding novel antimicrobial medications are still being created and are utilized extensively. Following the "golden era" revolution, during which almost all major antibiotic groups (including macrolides, tetracyclines, cephalpsporins and aminoglycosides) were found and the primary challenges of therapy were overcome in the 1960s, the historical pattern is being repeated now, with the rise in microbial resistance putting these widely used drugs at risk of becoming less effective. Even now, one of the main sources of novel therapeutic compounds is still natural products, primarily plants[3]. Worldwide, a vast range of infections are caused by bacteria.

Nonetheless, the pervasive and careless prescription of antibiotics has caused a proliferation of bacteria that were resistant to all standard medications. One ongoing issue is the rise of multiple medication resistance. Even now, *Staphylococcus aureus* continues to be among the most frequent infections seen in clinical settings, leading to a variety of serious illnesses. Because of the developing trend of medication resistance, bacteria like *Escherichia coli* and *Staphylococcus aureus* are a persistent issue in chemotherapy.

The rising incidence of *Escherichia coli* strains resistant to antibiotics exacerbates these diseases. A persistent demand exists for novel, safe and efficacious therapeutic medicines, given the concerning prevalence of antibiotic resistance in medically significant bacteria[10]. As a result of treatment failures brought on by the multidrug-resistant bacteria, it is currently having a significant impact and has raised public health concerns globally.

Genetically varied, big, deciduous climbing plant, *T. cordifolia*, also known as "Guduchi" in Sanskrit, belongs in the family Menispermaceae. The plant grows in India's tropical and subtropical zones. Large, deciduous, widely spreading and climbing shrub *T. cordifolia* has multiple long, twining branches. Despite preferring a warm climate, the plant can be grown practically anywhere due to its extreme rigidity. Typically, planting takes place in July through August, the rainy season[7]. It grows well on a wide range of soil types. Aerial roots are thread-like, long, filiform, squarish roots that grow downward from severed branches or mature branches and occasionally reach the ground by continuously growing. This plant has a long, thick, filiform and slightly succulent stem that tends to climb. Out of the branches grow aerial roots.

This plant has membranous, simple, alternating leaves that are about 15 cm long and have a long, round, pulvinate, heart-shaped petiole that is partially and halfway twisted. At higher altitudes, there is a shrub with typical greenish yellow flowers. Female flowers are solitary, whereas male blooms grow in clusters. Fruits are fleshy and cluster of one to three seeds[16].

The perennial plant *C. roseus*, also known as Vinca rosea or Madagascar periwinkle, is native to Madagascar and Southern Asia and is commonly found in tropical regions[12]. It has expanded over the tropical and subtropical areas of India, growing wild throughout the plains and lower foothills of the country's northern and southern hills. It is an evergreen sub shrub or herbaceous plant that reaches a height of 32 to 80 cm. It boasts vibrant, deep greenery and blooms throughout the summer season. The naturally occurring blossoms have a violet "eye" in the center of them and are pale pink in look. Erect or accumbent sutures, up to one-meter-long are typically made of white latex. Green stems are frequently tinged with red or purple. The plant has oval leaves which are lengthy, petiolate and decussate. With a mucronate apex, the lamina is varied, elliptic, obovate, or narrowly obviate.

Elegant white or pink flowers blossom with a center of pale yellow, crimson, purple, or white. The follicles are susceptible on the axial side. Flowering period is from spring to late falling in warm temperate areas and all year round in equatorial conditions. It is best to have full sun and well-drained soil. About 130 alkaloids are produced by it, mostly raubasine, vinceine, reserpine, vincristine and ajmalicine. Numerous cancers, including skin cancer, breast cancer, lymphoblastic leukemia and Hodgkin's disease, are treated with vincristine and vinblastine[18]. It has to be preserved because it is an endangered species via methods such as micropropagation. However, the purpose of the current investigation was to potentially affect the comparative study that assessed the anti-diabetic and anti-bacterial properties of *T. cordifolia* and *C. roseus* plants extract.

Material and Methods

Collection of plants sample from the different areas of District Hamirpur: The medicinal plants i.e. *T.cordifolia* and *C.roseus* were gathered from the Tihra Sujanpur Block in the Hamirpur, Himachal Pradesh. To get rid of dust and other foreign particles, the gathered plants was properly cleaned with tap water.

Preparation of plant extracts from both the plants *T. cordifolia* and *C. roseus*: *T. cordifolia* and *C. roseus* plant samples were obtained. After a thorough washing, the plants were chopped into small pieces and allowed to dry in the shade. Using a motor pestle, dry plants sample were crushed and then suspended in methanol at a 1:15 (w/v) ratio of sample to solvent for 48 hours. The methanolic plants extract underwent filtering after 48 hours of incubation. The extracts were stored in the refrigerator until they were needed again.

Tests for Carbohydrates: 1 ml of water was mixed with 0.5 mg of plants extract. The formulation turns yellow when an aqueous NaOH solution is added, signifying the presence of glycosides.

Tests for Protein: A few drops of HNO_3 were added to the extracts, the protein content was indicated by the yellow hue.

Tests for Alkaloids: Wagner's reagent in small amounts (one or two drops) was applied to 10 mg of extracts. Reddish-brown color changes revealed the existence of alkaloids.

Tests for Flavonoids: Drops of 8% lead acetate solution were combined with 10 mg of plants extract, yellow precipitates appeared. This suggests that flavonoids are present.

Tests for Phenols: 5% aqueous ferric chloride in 5 ml of water was added to the plants extract if it has a blue or green hue. This suggested the existence of phenols.

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Tests for Tannins: 0.5 ml of 5% $FeCl_2$ was combined with 5 mg of plant extract. The dark blue-black hue of the extract sample indicated the presence of tannins.

Tests for Sterols: Concentrated sulfuric acid was applied to 5 mg of leaf extract that has been dissolved in chloroform, carefully adding it along the test tube's edges. Sterol presence was shown by an appearance of red precipitate.

Anti-bacterial activity of *T. cordifolia* and *C. roseus*: The anti-bacterial activity of plants or microbial extracts was frequently assessed using the agar well diffusion method. The agar plate surface is inoculated by covering the whole surface with a volume of the microbial inoculum, a process that is similar to that employed in the disk-diffusion method. Subsequently, a sterile cork borer or tip is used to punch an aseptic hole measuring 6 to 8 mm in diameter. A volume of 30 and 50 µL of the antimicrobial agent or extract solution at the specified concentration is then added to the well. After that, agar plates are incubated in the appropriate environment for the test microorganism. The microbial strain under observation is inhibited from growing by the plant extracts and antibiotic as it diffuses throughout the agar medium[2].

Anti-diabetic activity of *T. cordifolia* and *C. roseus*: The anti-diabetic activity of both the plants extracts is frequently assessed using the α-amylase inhibitory assay. 1% starch solution, 100µl of 0.2 mm phosphate buffer (pH-6.9) and α-amylase (0.5 mg/ml) were added to samples of both plants extract at varying concentrations (100-500µg/ml). 2ml of the 3,5 dinitro alicyclic acid reagent were added to terminate the reaction after five minutes at 37°C. The reaction mixture was diluted in an ice bath using 10 ml of distilled water after being heated to 100°C for 15 minutes. The activity of α-amylase was determined by measuring the color intensity at 540 nm using a spectrophotometer. The equation can be used to calculate the inhibition of α-amylase[19].

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

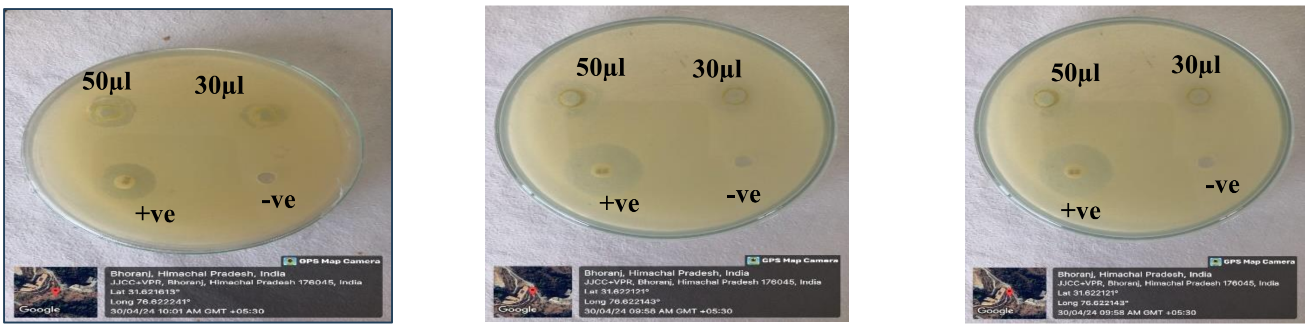
Results

Phytochemical screening of *T. cordifolia* and *C. roseus*: The results of phytochemical screening applied to the whole-part methanolic extracts of *T. cordifolia* and *C. roseus* are shown in table 1. As a result of this study, it was found that *C. roseus* includes phenols, sterols, proteins, alkaloids, flavonoids and tannins whereas *T. cordifolia* contains all of the above compounds expect sterols.

Anti-bacterial activity of *T. cordifolia* and *C. roseus*: The findings of the antibacterial activity of the methanolic extracts of *T. cordifolia* and *C. roseus* at concentrations ranging 30 and 50 µl (30 mg/ml) are displayed in table 2. The concentration of the methanolic extracts utilized in this investigation affects their antibacterial properties. *T. cordifolia* extract showed the anti-bacterial activity on *S. aureus* (MTCC-739, *E. coli* (MTCC-739) and *B. subtilis* (MTCC-441). Similarly, *C. roseus* extract inhibited the anti-bacterial activity of *S. aureus*, *E. coli* and *B. subtilis*. The standard medication for antibacterial activity was tetracycline.

Table 1
Phytochemical screening of the whole part of *T. cordifolia* and *C. roseus* plant extracts.

Phytochemicals compounds	*T. cordifolia* plant	*C. roseus* plant
Carbohydrates	+ve	+ve
Proteins	+ve	+ve
Alkaloids	+ve	+ve
Flavonoids	+ve	+ve
Phenols	+ve	+ve
Tannins	+ve	+ve
Sterols	-ve	+ve



A) *Staphylococcus aureus* (MTCC-737) **B) *Escherichia coli* (MTCC-739)** **C) *Bacillus subtilis* (MTCC-441)**

Fig. 1: Plant extracts of *T. cordifolia* showed zone of inhibition against both Gram-positive and Gram-negative bacterial strains.

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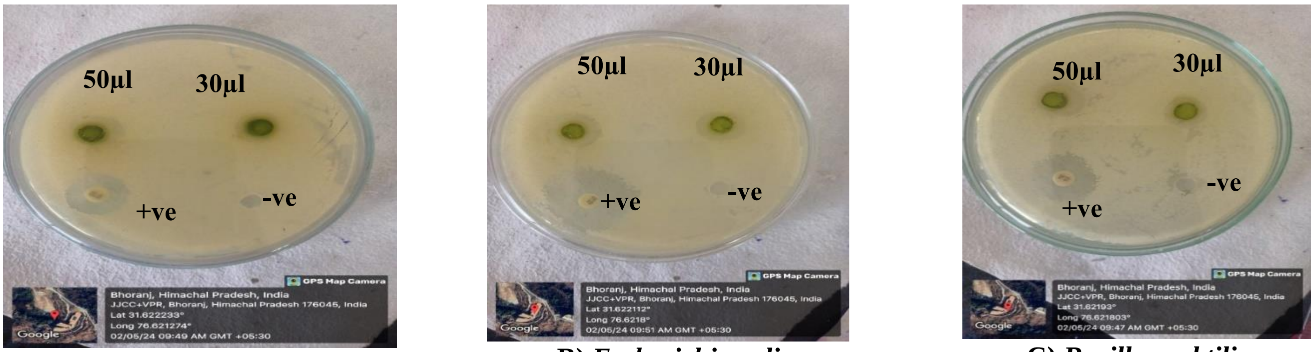

A) ***Staphylococcus aureus*** **(MTCC-737)** B) ***Escherichia coli*** **(MTCC-739)** C) ***Bacillus subtilis*** **(MTCC-441)**

Fig. 2: Plant extracts of *C. roseus* showed zone of inhibition against both Gram-positive and Gram-negative bacterial strains.

Table 2
Anti-bacterial activities of methanolic extract of *T. cordifolia* and *C. roseus*

		ZOI (mm)					
S.N.	**Microbial Strains**	***T. cordifolia* (30 µl)**	***T. cordifolia* (50 µl)**	**Tetracycline**	***C. roseus* (30 µl)**	***C. roseus* (50 µl)**	**Tetracycline**
1.	*Staphylococcus aureus* (MTCC-737)	12mm	15mm	16mm	10mm	15mm	15mm
2.	*Escherichia coli* (MTCC-739)	10mm	15mm	23mm	15mm	17mm	25mm
3.	*Bacillus subtilis* (MTCC-441)	10mm	15mm	18mm	10mm	15mm	20mm

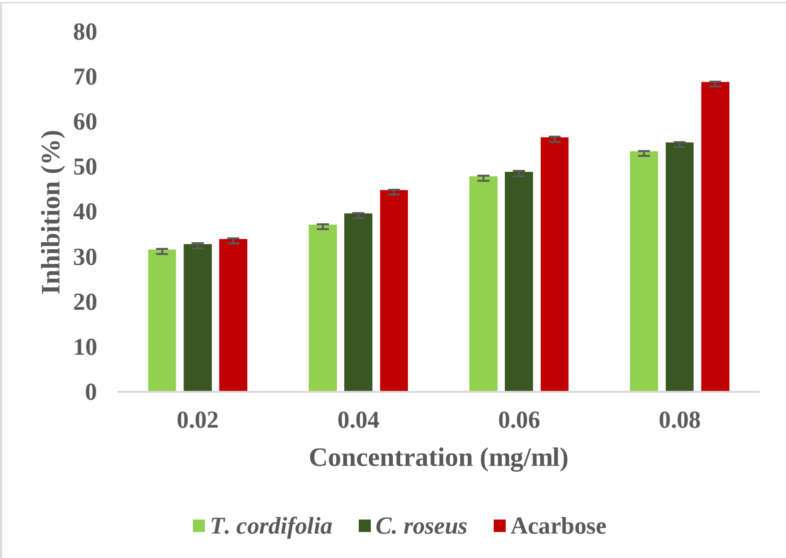

Fig. 3: α- amylase activity of Acarbose and extracts of *T. cordifolia* and *C. roseus* plants. Values are mean ± standard deviation of three replicates.

The maximum zone of inhibition (ZOI) was shown for *C. roseus* extract. Thus, *C. roseus* extract showed a greater anti-bacterial activity than *T. cordifolia* extract.

Anti-diabetic activity of *T. cordifolia* and *C. roseus*: Ayurvedic medicine uses a variety of herbal extracts that have been demonstrated to have antidiabetic effects to treat diabetes. In the *in vitro* α-amylase enzyme inhibition assay, the methanolic extracts of the whole plant extracts of *T. cordifolia* and *C. roseus* showed significant anti-diabetic activity. The α-amylase inhibitory activity of *T. cordifolia* (53.44%) extracts was lower than that of *C. roseus* extracts (55.43%) and standard. Acarbose (68.88%). Therefore, extracts from *C. roseus* plants showed higher anti-diabetic activity than extracts from *T. cordifolia* plants and they have a better potential to serve as an alternative source for the pharmaceutical industry.

Discussion

The majority of the world's traditional healthcare systems have included plant-derived medications for thousands of years and there is growing interest in herbs as a source of antimicrobial agents. The entirety of the *T. cordifolia* and *C. roseus* plants has been shown to offer important medicinal qualities in earlier research. Above study revealed the significant medical benefits for the whole extracts of *T. cordifolia* and *C. roseus* plant species. The study demonstrated the results of phytochemical screening of whole-part methanolic extracts of *T. cordifolia* and *C. roseus*. *C. roseus* contains phenols, proteins, alkaloids, sterols and flavonoids whereas *T. cordifolia* contains alkaloids, phenols, flavonoids, tannins and proteins, with the exception of sterols.

In contrast, *S. aromaticum* leaf extracts reveal the presence of alkaloids, diterpenes, flavonoids, glycosides, anthraquinones, saponins, coumarins, resins and oils. Its phytochemical content suggests that the plants extract may have therapeutic value and may be used in medicine and nutraceuticals[1]. Comparably, studies were conducted on the antibacterial activity of *T. cordifolia* and *C. roseus* methanolic extracts at concentrations ranging from 30 and 50 µl. The results indicated that *C. roseus* extract exhibited higher antibacterial activity than *T. cordifolia* extract[1]. For many bacterial strains, the *S. aromaticum* plant extract shows the zone of inhibition. For *E. Coli*, the extract displayed a zone of inhibition (ZOI) of 19 mm, while the zone of streptomycin, the standard antibiotic, was 22 mm.

With a ZOI of 18 mm for *B. subtilis*, the extract was somewhat less potent than the common antibiotic streptomycin, which had a ZOI of 22 mm. On the other hand, the ZOI of the *S. aureus* plant extract was 13 mm. *In vitro* α-amylase enzyme inhibition experiment showed significant anti-diabetic activity of the methanolic extracts of the entire plants of *T. cordifolia* and *C. roseus*. Compared to *C. roseus* extracts (55.43%) and standard acarbose (68.88%), *T. cordifolia* extracts had a weaker inhibitory effect from α-amylase (53.44%). According to Egharevba et al[4], an α-glucosidase enzyme inhibition assay conducted *in vitro*, revealed that the TbHL and TbEAL extracts of *T. bracteolate* leaves showed modest anti-diabetic activities.

α-glucosidase enzyme percentage inhibitory activity (67%) was observed higher in the TbEHL extract of *T. bracteolata*, in the TbAL extract as 57% and in standard acarbose as 92.95%. Therefore, extracts from *C. roseus* plants showed higher anti-diabetic action than extracts from *T. cordifolia* plants and they have a better potential to serve as an alternative source for the pharmaceutical industry.

Conclusion

The findings showed that whole plant extracts from *T. cordifolia* and *C. roseus* were found to include phenols, flavonoids, proteins, carbohydrates and sterols respectively. Promising anti-bacterial and anti-diabetic activities were shown by the both plant extracts in methanol. *T. cordifolia* and *C. roseus* methanolic extracts showed a maximal zone of inhibition against *Staphylococcus aureus* (MTCC-737) and *Escherichia coli* (MTCC-739) respectively. Maximum zone of inhibition was seen in *C. roseus* relative to *T. cordifolia*. *C. roseus* extracts having a stronger α-amylase inhibitory activity (55.43%) compared to *T. cordifolia* extracts (53.44%).

Based on this study, the pharmaceutical or herbal drug industries may benefit most from extracts of the *T. cordifolia* and *C. roseus* plants. Our findings validate the use of *in vitro* grown plants as a viable substitute for fulfilling the growing needs of the pharmaceutical sector, creating several opportunities for the development of herbal medicinal formulations, given the significant decline in the wild population of this herb.

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