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Research Article

Phytochemical and Antimicrobial Analysis of Methanol Extract of Stem of *Tridax procumbens*

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Abstract

The present study aims to evaluate the phytochemical and antimicrobial activity of methanol extract of stem of *Tridax procumbens* against some selected bacteria (*E. coli, S. enterica typhimurium, S. flexneri, S. aureus*). A cold extraction method was issued to obtain the methanol extract from stems of *Tridax procumbens.* The antimicrobial activities of this extract were studied on the various test microorganisms using the agar well diffusion technique. phytochemical analysis of this extract was done by using chemical methods. Antimicrobial activity of acetone extract from stem of *T. procumbens* against *E. coli* was 5.0 + 0.55 mm, *S. enterica typhimurium* 7.0 + 0.82 mm, *S. flexneri* 16 + 1.2 mm, and *S. aureus* 4 + 0.5 mm respectively. Phytochemical analysis showed the presence of chemicals like alkaloids, flavonoids, steroids, and tannins. Methanol extract of the stem of *Tridax procumbens* showed antimicrobial activity against all the selected bacteria.

Keywords: Antimicrobial Activity; Phytochemical Analysis; *Tridax procumbens*; Methanol Extracts

Introduction

Antibiotics are failing to provide protection to humans due to the emergence of antibiotic-resistant microorganisms. Thus, there is a need for safer and more effective antimicrobial agents. Since ancient times, people are using plant-based medicine. They not only provide protection against pathogens but also increase the immunity to fight against pathogens. *T. procumbens* is also used since ancient times for medicinal purposes.

The phytochemical tests showed the presence of alkaloids, flavonoids, phenols, saponins, steroids, and tannins in the methanolic extract of *Tridax procumbens* (METP) [1]. The extracts of *Tridax procumbens* have been reported to have various pharmacological effects like mosquito repellent activity, leishmanicidal, hepatoprotective effect on the liver antioxidant system, immunomodulatory effect; wound healing activity, and antiprotozoal effects [2].

T. procumbens is also being studied for naturally occurring antioxidants and antibacterial agents for use in food or medicinal materials [2,3]. This plant's ethanol and methanol extracts have been shown to have antioxidant activity [4]. This weed's aqueous, ethanol, and methanol extracts were antibacterial against *E. coli* [5,6,], while its ethyl acetate extract was antibacterial against *Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae, E. coli,* and *Bacillus cereus* [2].

According to existing reports, *T. procumbens* has been one of the most important medicinal plants used for its anti-ailment effects from ancient times to the present. To explore more pharmacological properties of the plant a phytochemical screening and antimicrobial analysis of the methanolic extract of the stem were done in the present study.

Materials and Methods

Collection and processing of the plant

T. procumbens were collected from the area near the Christ College campus, Rajkot. All the plants were identified by Dr. Jignasa Joshi, working as an Assistant Professor at the Department of Biology at Christ College in Rajkot. Whole plants were taken washed with water and then rinsed with Distilled water. Different parts of these plants were separated and allowed to air-dry for several days. Dried stems were powdered with the help of a mechanical grinder. The prepared powder was kept in an airtight bottle for further use [7].

Preparation of extract

For the preparation of extract, methanol was used. The extract was prepared by taking 100 grams of powder from stems of *T. procumbens* in 300 ml of methanol. This mixture was kept on an orbital shaker at 40 rpm for 3 days. After 3 days the mixture was filtered using Whatman filter paper No. 1. The filtrate was allowed to evaporate by air drying. The extract was preserved in the refrigerator for further use [8]. The stock solution of the extract was prepared by taking 100 milligrams of extract per ml of dimethyl sulfoxide (DMSO) [9]. The stock solution was preserved in the refrigerator at 4°C. The extraction yield of selected plants has been calculated by the following equation [10].

The Yield. % = X1 x 100/X0

Where X1 denotes the weight of the extract after solvent evaporation and X0 denotes the dry weight of the plant powder prior to extraction.

Phytochemical analysis

Phytochemical tests for analyzing different chemical compounds present in the extract were performed as per the method given by [11].

- **Test for Steroids:** Two ml of extract was dissolved in chloroform; 2ml of concentrated sulphuric acid was added to the mixture. The red color formation indicates the presence of steroids.
- **Test for Glycosides:** Two ml of extract was dissolved in chloroform and 2 ml of acetic acid was added to the mixture. The solutions were cooled and then a few drops of sulphuric acid were added. The presence of Glycosides is indicated by a change in color from blue to green.
- **Test for Flavonoids:** To 1 ml of extract, 1 ml of 10% lead acetate was added, formation of a yellow precipitate indicates the presence of flavonoids.
- **Test for Saponins:** Two ml of extract was shaken vigorously with 5 ml of distilled water and warmed. The Stable foam formed to indicate the presence of saponins.
- **Test for Tannins:** 2 ml of extract is diluted with 2 ml of distilled water and thoroughly mixed. The formation of green precipitate after the addition of a few drops of ferric chloride showed the presence of tannins.
- **Test for Amino acids:** One ml of extract was treated with a few drops of ninhydrin reagent. The presence of amino acids will be indicated by the purple color of the mixture.
- **Test for Alkaloids:** Three ml of extract was added to 1% HCL and then allowed to the steam bath. A few drops of Mayer & Wagner's reagent were added to the mixture Turbidity indicates the presence of alkaloids.

Selection of bacterial cultures

Bacteria like *Escherichia coli* (MTCC 443), *Salmonella enterica typhimurium* (MTCC 98), *Shigella flexneri* (MTCC1457), and *Staphylococcus aureus* (MTCC 3160) were procured from MTCC Chandigarh. These bacteria are stored on N-Agar slant (Hi-media) at 4°C for further use.

Preparation of inoculum

In a rotary shaker at 37°C, all of the bacteria were pre-cultured in Mueller Hinton broth (MHB) for 24 hours. Following that, using the 0.5 McFarland standard, each strain was adjusted to a concentration of 10^8 cells/ml [12].

Antimicrobial analysis

Bacterial cultures were inoculated into N broth for 18 h at 37°C. The antimicrobial analysis was performed by the agar well diffusion method. Plates of Mueller - Hinton agar (Himedia) were prepared and allowed to solidify. Bacteria were inoculated on plates using a sterile swab. Well has been prepared with the help of a sterilized cup-borer 8 mm in diameter. 0.1 ml of extract dissolved in DMSO was poured into the well. Plates were incubated in an incubator

at 37°C for 24 h. After 24 h zones of inhibition were observed and measured in mm [13].

Result and Discussion

Percentage yield extract

In the methanol extract of the stem of *T. procumbens*, the percentage yield of the extract was found to be 7.0%. The number and quantity of phytochemicals present in the extract will determine the bioactivity including the antibacterial activity of the extract. More the yield of the extract may give more activity but it may not be true as the compounds present may be antimicrobial or may enhance the growth of the microbes.

Phytochemical analysis

Phytochemical analysis of the methanol extract of the stem of T. *procumbens* showed the presence of Alkaloids, Flavonoids, Steroids, and Tannins as tabulated in table 1. Phytochemical constituents such as alkaloids, flavonoids, glycosides, and several other aromatic compounds are secondary metabolites in plants that have alleviated the pathogenic and environmental stress [14]. Thus, the presence of these compounds in the extract signifies the antibacterial activity of the extract.

Phytochemical constituents	Result
Alkaloids	+
Flavonoids	+
Saponins	-
Steroids	+
Tannins	+
Glycosides	-
Amino acids	-

Table 1: Preliminary phytochemical evaluation of methanol extracts stems of *Tridax procumbens.*

(+) = Present; (-) = Absent

Antibacterial activities

Antibacterial activity of methanol extract from the stem of *T. procumbens* against selected bacteria showed in table 2. Antibacterial activity of this extract was found against all the selected bacteria. Maximum activity was against *S. flexneri* (16.0 ± 1.2), while the least activity was against *S. aureus* (4.0 ± 0.5). Thus, gram-positive bacteria were less affected while gram-negative were more affected. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with no or lesser side effects due to an array of secondary metabolites [15].

Bacteria	Mean Zone of inhibition + SD (in mm)
E. coli	5.0 ± 0.55
S. enterica typhimurium	7.0 ± 0.82
S. flexneri	16.0 ± 1.2
S. aureus	4.0 ± 0.5

Table 2: Antibacterial evaluation of methanol extracts stems of *Tridax procumbens.*

Mean value of triplicate + standard deviation.

Conclusion

The presence of numerous bioactive compounds in the methanolic extract of *Tridax procumbens* stem confirms that this part of the plant has significant potential. They can be studied further to see if they can be used to treat infectious diseases, inflammatory diseases, cancer, arthritis, coronary diseases, immuno-modulation, and neurological disorders. Isolation of individual phytochemical constituents and further investigation of their biological activity, on the other hand, will yield more fruitful results. As a result, additional research is required to identify and purify the active compounds responsible for the therapeutic activity.

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Conflicts of Interest

The authors declare no conflict of interest.

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