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# STUDIES ON ANTIOXIDANT ACTIVITY AND ANTIMICROBIAL ACTIVITY OF *Dipsicus inermis*

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**Abstract**

Natural products with medicinal value are gradually gaining importance in clinical research due to their well-known property of no side effects as compared to synthetic drugs. *Dipsicus inermis* (Willd.) commonly named as "Guduchi" is known for its immense application in the treatment of various diseases in the traditional Ayurvedic literature (Saha and Ghosh., 2012).

In the present study the aqueous, methanol, ethanol and acetone extract of *Dipsicus inermis*, stem extract was screened for the presence of phytochemical components and tested for antifungal activity against five pathogens such as *Candida albicans, Aspergillus niger, Aspergillus fumigatus, Microsporum gypseum and Trichophyton rubrum* results revealed the presence of alkaloids, flavonoids, saponins, tannins, glycosides, Steroids, Anthraquinones and phenolic compounds. The acetone extracts had wide range of antibacterial activity against bacterial pathogens than the ethanol and methanol extract, where as aqueous extract were slightly higher antibacterial activity as ethanol extract. Antifungal activity of stem extract of *Dipsicus inermis,* would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

**Key words:** Phytochemical Screening, Antifungal activity, *Dipsicus inermis*, stem extract.

## Introduction

A large number of plants are being used in medicine for therapeutic or prophylactic purposes. The therapeutic properties of medicinal plants are attributed owing to the presence of active substances such as alkaloids, flavonoids, saponins, tannins, glycosides, Steroids, Anthraquinones and phenolic compounds. These natural compounds physiologically affect the body of human beings, interact with the pathogens and interrupt their growth at different stages of development and make the body disease free.

Young stems of *Tinospora cardifolia* green with smooth surfaces and swelling at nodes, older ones show a light brown surface marked with warty protuberances due to circular lenticels, transversely smoothened surface shows a radial structure with conspicuous medullary rays traversing porous tissues, taste bitter (Fig-1).

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (Rastogi and Mehrotra, 2002). Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.,* 1999). Emergence of multidrug resistant pathogens has been reported to be one of the leading causes of death world (Reddy *et al.,* 2009). wide with infectious diseases responsible for 68% of all deaths globally in 2012 (WHO, 2000). Many infectious microorganisms' are resistant to synthetic drugs and it has become the major concern for health institutions, pharmaceutical companies and governments all over the world; thus there is need for an alternative therapy (Tambekar and Dahikar, 2011).

It is widely used in Ayurveda for a variety of purposes associated with inflammation, allergies, neurology and glucose metabolism. The pharmaceutical significance of this plant is mainly stem and roots, which contain various bioactive compounds such as alkaloids, flavonoids, saponins, tannins, glycosides, Steroids, Anthraquinones and phenolic compounds, having various medicinal importances. Some of the important applications are *viz*. immunomodulatory or immunostimulatory, anti-neoplastic, anti-oxidant, anti-hyperglycemia, anti-hyperlipidemia, antituberculosis, hepatoprotection, anti-osteoporotic, anti-angiogenic, anti-malarial and anti-cancer.

*Tinospora cardifolia* (Giloe) is perhaps the most useful traditional medicinal plant. Every part of the plant has been used as traditional medicine for household remedy against various human ailments. The tree is still regarded as "Village dispensary" in India. Most of the parts of the plant such as leaves, stem and root contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer and antifungal properties. It has made an important contribution to the modern research due to its large number of medicinal properties. Different parts of the plant have shown antimicrobial, anti-inflammatory, analgesic, antipyretic, antiulcer, antidiabetic, antioxidant and anticancer activity. *Tinospora cardifolia* is a large deciduous climbing shrub found throughout India.

The ayurvedic name of the plant is Guduchi, Giloe or Amrita. In India, the extract of the plant is used as a remedy for many diseases including diabetes, hepatitis etc., The plant finds a special mention for its use in tribal or folk medicine in different parts of the country. The drug has been subjected to extensive phytochemical, pharmacological and clinical investigations and many interesting findings have been reported (Nadkarni, 2005).

Many researchers had studied antimicrobial activity of other parts of plant like stem and root of *Dipsicus inermis*, which are used to cure many infectious diseases in traditional system of medicine but still very, less work has been done on antibacterial activities of stem of *Dipsicus inermis*. To prove the validity of traditional medicine the present work has been undertaken to evaluate the antimicrobial screening of stem of *Dipsicus inermis* against the human bacterial pathogens.

## Materials and Methods

### Sample Collection

Fresh plant of *Dipsicus inermis* were collected from G.B.Pant University of Agricultural & Technology, Pantnagar, (U.K). It was identified by Dept. of Medicinal and Aromatic Plants, G.B. Pant University of Agriculture And Technology, Pantnagar, Uttarakhand.

### Chemicals & Hi-Media

Chloroform, hexane, methanol, Acetone, ethanol, ferric chloride, magnesium, petroleum ether, ethyl acetate, HCL, $H_2SO_4$, glacial acetate, Mayer's reagent, ammonia chloride, were from Merck Uttarakhand, (India).

Sabouraud dextrose media, Potato Dextrose broth, were from Hi-media laboratories, Mumbai, India.

### Preparation of plant material

Stem was collected and dried at room temperature. The dried samples were powdered separately. 100gm each of the sample was extracted separately with different solvents starting with polar to non polar solvents in the order of aqueous, ethanol, methanol and acetone. The crude residues were obtained by removing the solvents in rotary evaporator and each of the extracts were resuspended in the respective solvents for further study.

**Preparation of extracts:** Solvent extraction method 30 gm of dried powder of *Dipsicus inermis*,

stem was extracted with aqueous, ethanol, methanol and acetone using soxhlet apparatus for 48 hrs. The collected extracts were filtered with Whatman No.1 filter paper and used for estimation of phytochemicals and antibacterial activity.

**Phytochemical screening:** Phytochemical screening of *Dipsicus inermis*, stem extract qualitatively to test for the presence of alkaloids, flavonoids, saponins, tannins, glycosides, phenolic compound, Steroids and Anthraquinones were carried out with the following methods (Khandelwal, 2001).

**Test for Alkaloids:** To 4 ml of extract filtrate, a drop of Mayer‟s reagent was added along the sides of test tube. Creamy yellow or white precipitate indicates that the test is positive.

**Test for Flavonoids:** A volume of 1.5 ml of 50 % methanol was added to 4 ml of the extracts. The solution and magnesium metal was added and warmed. Then, 5 to 6 drops of concentrated hydrochloric acid was added to the solution and observed for red coloration.

**Test for Saponins:** Two ml of distilled water was added to 2 ml of the test solution shaken well and observed for frothing.

**Test for Tannins:** To 0.5 ml of extract solution, 1 ml of distilled water and 1 to 2 drops of ferric chloride solution was added, observed for blue or green black coloration.

**Test for Glycosides:** To 4 ml of extract solution and add few drops of glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid and observed for a reddish brown coloration at the junction of 2 layers and bluish green colour in upper layer.

**Test for phenolic compounds:** Two ml of extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5 % ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds

**Test for Steroids (Salkwoski's test):** Five drops of concentrated sulphuric acid (H2SO4) was added to 2 ml of each extract and observed for red coloration.

**Test for Anthraquinones:** 1 gm of powdered plant material was taken and extracted with 10 ml of hot water for five minutes and filtered. Filtrate was extracted with 10 ml of CCl4 then CCl4 layer was taken off. Five ml water and 5 ml dilute ammonia solution was added. No free anthraquinones were revealed as absence of appearance of pink to cherry red colour. 1 gm of second sample of the same plant material was extracted with 10 ml of ferric chloride solution and 5 ml of hydrochloric acid then it was heated on water bath for 10 minutes and filtered. Filtrate was cooled and treated as mentioned above.

**Antifungal activity using disc diffusion method:**

**Fungal cultures:** The standard pathogenic fungal cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The fungal culture rejuvenated in Sabouraud dextrose media, (Hi-media laboratories, Mumbai, India) incubate at 37 °C for 18h and then stocked at 4 °C in Sabouraud Dextrose Agar (SDA). Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 ml of sterile Potato Dextrose broth and incubated at 37 °C for 24h. Turbidity of the culture was standardized to $10^5$ CFU with the help of Turbidometer.

**Table 1:** Fungal cultures used in study (IMTECH, Chandigarh, India).

| Sr.No. | Fungal Cultures | MTCC Number |
|---|---|---|
| 1. | *Candida albicans* | 183 |
| 2. | *Aspergillus niger* | 478 |
| 3. | *Aspergillus fumigates* | 870 |
| 4. | *Microsporum gypseum* | 7675 |
| 5. | *Trichophyton rubrum* | 296 |

The modified paper disc diffusion was employed to determine the antifungal activity of solvent extract of stem of *Dipsicus inermis*. For antifungal properties, 0.1 ml fungal suspension of $10^5$ Cfu/ml was uniformly spread on Potato Dextrose Agar (PDA) plate to form lawn cultures. The petroleum ether, chloroform, ethyl acetate and methanol extracts were prepared in their respective solvents in such a manner that ultimate amount (in dry form) in each disc came to 10mg, 8mg, 6mg, 4mg and 2mg. The blotting paper discs (10mm diameter) were soaked in various diluted extract, dried in oven at 60 °C to remove excess of solvent and tested for their antifungal activity against fungal pathogens by disc diffusion technique. After incubation of 24 h at 37 °C, zone of inhibition of growth was measured in mm. The antifungal activity was classified according to the zone of inhibition such as strong (19-22mm), moderate (15-18mm) and mild (11-14mm). Griseofulvin 10mcg (Hi-Media disc) was used as positive control while discs soaked in various organic solvents and dried were placed on lawns as negative control.

**Results and Discussion**

Phytochemical screening revealed presence of alkaloids, flavonoids, saponins, tannins, glycosides and phenolic bioactive compound suggesting it to be a potential medicinal plant.

The medicinal plant of Giloe, is being used traditionally for the treatment of inflammation, wound healing, carminative, cough, toothache, antiseptics expectorant, stomatitis and some fungal infection like candidaisis. In this context, natural, multifunctional, stable, non-toxic and natural compounds from plants which are pharmacologically effective or with low or no side effects are preferred for use in preventive Ayurvedic medicine and in food industry (Sati *et al*., 2010).

The antibacterial activity has been attributed to the presence of some active constituents in the extracts. The phytochemical analysis of *Dipsicus inermis* extract had earlier been reported. Phytochemical screening of the stem extract of *Dipsicus inermis* in the present study also revealed presence of alkaloids, flavonoids, saponins, tannins, glycosides, Steroids, Anthraquinones and phenolic compounds. Study suggested a number of active constituents might be present in the *Dipsicus inermis*, stem extract to control gastroduodenal ulcers.

However, a glycoside appeared to be the major bioactive component that offers antisecretory and antiulcer effects. Plant glycosides, which are not normally toxic when ingested orally, are known to inhibit chloride transport in the stomach. Phytochemical screening of the stem extract of *Dipsicus inermis* in the present study also revealed presence of alkaloids, flavonoids, saponins, tannins, glycosides and phenolic compounds.

**Table 2:** Phytochemical analysis of stem extract of *Dipsicus inermis.*

| Sr.NO. | Phytochemical Constitutes | Aqueous Extract | Ethanol Extract | Methanol Extract | Acetone Extract |
|---|---|---|---|---|---|
| 1. | Alkaloids | +ve | +ve | +ve | +ve |
| 2. | Flavonoids | +ve | +ve +ve | +ve +ve | +ve +ve +ve |
| 3. | Glycosides | +ve | +ve | +ve | +ve |
| 4. | Saponins | -ve | +ve +ve | +ve +ve | +ve |
| 5. | Steroids | -ve | +ve | +ve | +ve |
| 6. | Tannins | +ve | +ve +ve | +ve +ve +ve | +ve +ve +ve |
| 7. | Anthraquinones | -ve | +ve | +ve | +ve |
| 8. | Phenolic compounds | -ve | +ve +ve +ve | +ve +ve +ve | +ve +ve +ve |

-ve : absent, +ve: present in low concentration, +ve+ve: present in moderate concentration, +ve +ve +ve: present in high concentration.

**Table 3:** Antifungal activity of *Dipsicus inermis*, stem extracts against fungal pathogens (Zone of inhibition of growth in mm, average of 3 readings).

| Plant | Solvent Extract | *Candida albicans* | *Aspergillus niger* | *Aspergillus fumigatus* | *Microsporum gypseum* | *Trichophyton rubrum* |
|---|---|---|---|---|---|---|
| Dipsicus inermis | Aqueous | 25 mm | 20 mm | 29 mm | 6 mm | 24 mm |
| | Ethanol | 22 mm | 18 mm | 27 mm | 18 mm | 26 mm |
| | Methanol | 26 mm | 24 mm | 29 mm | 22 mm | 28 mm |
| | Acetone | 26 mm | 28 mm | 29 mm | 23 mm | 28 mm |
| Positive control | Griseofulvin (10mcg) Hi-media disc. | 30 mm | 25 mm | 22 mm | 18 mm | 20 mm |
| Negative Control | Water | -ve | -ve | -ve | -ve | -ve |
| | Ethanol | -ve | -ve | -ve | -ve | -ve |
| | Methanol | -ve | -ve | -ve | -ve | -ve |
| | Acetone | -ve | -ve | -ve | -ve | -ve |

According to Antifungal activity of *Dipsicus inermis*, stem extracts against fungal pathogens (Zone of inhibition of growth in mm, average of 3 readings) Table 3, shown the petroleum ether extract exhibited strong inhibitory activity against *Candida albicans* and *Aspergillus niger*, but had a moderate antifungal activity against *Aspergillus fumigatus*, *Microsporum gypseum* and mild antifungal activity against *Trichophyton rubrum.* Chloroform extract showed moderate antifungal activity against *Candida albicans*, *Aspergillus fumigatus*, and mild antifungal activity against *Aspergillus niger*, *Microsporum gypseum*, *Trichophyton rubrum.* Ethyl acetate extract showed moderate antifungal activity against *Candida albicans*, *Aspergillus fumigatus*, and mild antifungal activity against *Aspergillus niger*, *Microsporum gypseum* and *Trichophyton rubrum.*

Ethanol extract showed strong antifungal activity against *Aspergillus fumigatus, Trichophyton rubrum,* but moderate antifungal activity against *Candida albicans* and mild antifungal activity against *Aspergillus niger, Microsporum gypseum.* Methanol extract showed strong antifungal activity against *Aspergillus fumigatus, Trichophyton rubrum* but had moderate antifungal activity against *Candida albicans* and mild antifungal activity against *Aspergillus niger, Microsporum gypseum.*

Plant extracts of *Dipsicus inermis* have been reported to have potential against microbial infections. The anti-bacterial activity of *Dipsicus inermis* stem extracts has been assayed against various Gram positive and Gram negative organisms. The antimicrobial activity of *Dipsicus inermis* stem extracts was investigated against bacteria causing UTIs viz. uropathogens, Escherichia coli and Staphylococcus aureus.

The study conducted using disc diffusion method showed that all three solvent extracts of *Dipsicus inermis* reveal different antibacterial activity against both uropathogenic isolates with decreasing order as ethanolic (maximum) > methanolic (moderate) > aqueous (poor) (Priyanka *et al*., 2015). The larger zones of inhibition exhibited by *Dipsicus inermis stem* extract. against *A. niger* may be due to the presence of variety of active compounds. This is well known, since tannins and saponins are important plant metabolites which is responsible for their antimicrobial activity. From the results obtained, the stem extract of *Dipsicus inermis* showed antifungal activity among the entire fungal organism. This suggests that *T. cordifolia* contains more of the active compounds and has high potency. In the present study the biological activity of the acetone extract of *Dipsicus inermis* can be attributed to the synergistic effect of the combination of flavonoids, steroids, terpinoids and saponins. Phenolic compounds are widely found in the secondary products of medicinal plants, as well as in many edible plants (Hagerman *et al*., 1998).

**Conclusion**

In present study, *Dipsicus inermis* has shown better potential antimicrobial activity.suggests that the identified phytochemicals may be the bioactive constituents responsible for the efficacy of stem extract of *Dipsicus inermis*. It may be concluded from this study that *Dipsicus inermis* stem extract has potential antimicrobial activity. This can explain that the host plant will gain some of the activities when they survive on medicinal plants. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

The results obtained in this study reveals the different potential antimicrobial activity manipulations in prevention or control of diseases. Natural products from dietary components such as Indian species and medicinal plants are known to possess antimicrobial activity.

it is concluded that phytochemical components as flavonoids, saponins, tannins, glycosides, Steroids, Anthraquinones and phenolic compounds of *Dipsicus inermis,* stem extract responsible for antimicrobial activity.

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