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Antifungal activity and Phytochemical screening of Tinospora cordifolia

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Abstract:

Various extracts of solvent of *Tinospora cordifolia* (family- Menispermaceae) leaves such as hexane, chloroform, dichloromethane, ethyl acetate, methanol and aqueous were evaluated for their phytochemical and antifungal activity against selected fungal sps.like *Aspergillus niger, Aspergillus fumigatus, Candida albicans, Saccharomyces cerevisiae and Microsporum gypseum* by paper disc diffusion method. The activities of the samples were compared with that of standard antibiotics 'Griseofulvin.' Phyto-chemical screening of *Tinospora cordifolia* leaf extract in five different solvents showed the presence of important Phyto-constituents like phenols, alkaloids, flavonoids, tannins and saponins. In antifungal assay, all the organisms respond to the plant extract but inhibitory zone developed. The chloroform extract showed a higher activity than other extracts. The methanolic extract of *T. cordifolia* showed higher activity against *A. fumigatus* and *A. niger* followed by, *Candida albicans, Saccharomyces cerevisiae and Microsporum gypseum* the methanolic extract of *T. cordifolia* showed maximum zone of inhibition against *A. fumigatus*. The ethyl acetate extract of *T. cordifolia* exhibited high activity against *A. niger* and *A. fumigatus* followed by methanol, chloroform, hexane, and aqueous extracts. The results revealed that the antimicrobial activity exhibited by *T. cordifolia*, chloroform and aqueous extract noted to be most effective than other solvents.

Key words: *Tinospora cordifolia*, leaves extracts, phytochemical Screening, antifungal activity

INTRODUCTION:

Tinospora cordifolia is a widely used shrub in folk and Ayurvedic systems of medicine (Rastogi and Mehrotra, 2002) It is distributed throughout the tropical Indian subcontinent and China, ascending to an altitude of 300 m. It is glabrous, deciduous climbing shrub belonging to the family Menispermaceae. *Tinospora cordifolia* grows as climber; leaves are simple, alternate, and long petiole, lamina broadly ovate, nerved and deeply cordate at base. Flowers grow during summer and fruits during winter. Stem of *Tinospora cordifolia* appears in varying thicknesses, young stems are green with smooth surfaces and swelling at nodes, while the older ones are light brown in colour with warty protuberances at the surface due to circular lenticels; transversely smoothened surface shows a radial structure with conspicuous medullary rays traversing porous tissues and is bitter in taste.

In Hindi, the plant is commonly known as Giloe which refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally young. Guduchi, its Sanskrit name, means one which protects the

entire body. The term amrita is attributed to its ability to impart youthfulness, vitality, and longevity. It is an annual or perennial Ayurvedic plant which is used in several traditional medicines to cure various diseases. The plant is sometimes cultivated for ornamental value and is propagated by cuttings. The leaves afford a good fodder for cattle. *Tinospora cordifolia* finds a special mention for its use in tribal or folk medicine in different parts of the country (Jain ,1991). Almost all the parts of the plant are documented to be useful in ethnobotanical surveys conducted by ethnobotanists (Singh et.al. 2003).

Tinospora cordifolia is reported to possess antispasmodic, anti-inflammatory and antiallergic properties. The notable medicinal properties reported are antidiabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, antistress, anti-leprotic, anti-malarial, antipyretic, hepatoprotective, immunomodulatory and antineoplastic activities (Nadkarni, 2005). The chemical constituents reported from this shrub belong to different classes such as alkaloids, terpenoids lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. The starch obtained from the stem known as Guduchi satva is highly nutritive and digestive and used in many diseases. Starch is present throughout the parenchyma of the stem. It is a tonic and useful in the treatment of chronic diarrhoea and dysentery. It is a hepato-protectant and protects the liver from damage. This is especially used when the liver has been exposed to various toxins.

MATERIALS AND METHODS:

Collection of Plant sample:

Fresh and healthy stem of *Tinospora cordifolia* were collected from in and around Degloor region randomly. The samples were washed with tap water to remove dust and contaminant. The plant samples were shade dried until all the moisture evaporated and pulverized by using mechanical grinder and stored in air tight jar for further use.

Extraction of plant material:

The plant materials were extracted with ethanol using Soxhlet apparatus continuously for 6 to 8 hours. 50 gm of dried plant material was packed in filter paper and loaded into the thimble of Soxhlet apparatus. 250 ml of different extract viz; aqueous, ethanol, methanol and acetone was poured into the flask and the all apparatus was set. The extraction was performed for 6-8 hours. Later the extracted solvent was evaporated under reduced pressure. Then the extract was kept in refrigerator for further use.

Phytochemical analysis

The qualitative analysis of tannins, phenols, glycosides, alkaloids, steroids, and flavonoids were analyzed by standard method (Harborne, 1973).

Selected test microorganisms

Extracts were tested against pathogenic microbes, including the fungi *Aspergillus niger, Aspergillus fumigatus, Candida albicans, Saccharomyces cerevisiae and Microsporum gypseum.*

Antifungal activity using disc diffusion method:

The modified paper disc diffusion was employed to determine the antifungal activity of solvent extract of leaves of *Tinospora cordifolia*. For antifungal properties, 0.1 ml fungal suspension of 10^5 CFU ml^{-1} was uniformly spread on 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 60^0 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 ^{0} 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 (Singh et.al. 1981).

Results and Discussion

Phytochemical screening of *Tinospora cordifolia*

Presence and absence of primary phytochemical viz., alkaloids, flavonoids, glycosides, steroids, phenols, tannins, saponins and resins was confirmed in the laboratory tests.

The preliminary phytochemical results of selected solvent extracts of *Tinospora cordifolia* were showed in the Table 1. It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds (Cook *et al.,* 1996). Flavonoids are most known for their antioxidant activity. They are modifiers which modify the body's reactions to allergens, viruses, and carcinogens (Korkina et. al. 1997). They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity (Priyanka et. al., 2015). The presence of alkaloids explains its anti-bacterial activity, since this phytochemical is reported to have anti-bacterial activity (Kher et. al 1977, Rhoades 1979, Idowu *et al.,* 2003). Tannins are reported to have various physiological effects like anti-irritant, antifungal and antimicrobial and antiparasitic effects. Phytotherapeutically tannin-containing plants are used to treat nonspecific diarrhea, inflammations of mouth and throat and antimicrobial.

Table.1 Preliminary phytochemical screening of selected solvent leaf extracts of *T. cordifolia*

S.No.	Phyto-chemicals	n-Hexane	Chloroform	Dichloromethane	Ethylacetate	Methanol	Aqueous
1.	Alkaloids	-	-	-	+	-	-
2.	Flavonoids	+	+	+	+	+	-
3.	Glycosides	+	+	+	-	+	+
4.	Steroids	-	-	-	-	-	-
5.	Phenols	-	+	+	+	+	+
6.	Tannins	-	+	+	+	+	+
7.	Saponins	+	-	-	-	-	-
8.	Resins	-	-	-	+	+	-

+ = Presence, - = Absence.

Antifungal activity of *T. cordifolia*

The antifungal activity has been screened because of its great medicinal relevance with the recent years, infections have increased to a great extent and resistance against antibiotics, become an ever-increasing therapeutic problem (Ali et al., 2001). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobial compounds. Table.2 represents the antifungal effect of selected solvent extracts of *T. cordifolia* by paper disc diffusion method against selected fungal strains and the zone of inhibition was assessed in millimeter diameter. The aqueous extract of plant showed best zone of inhibition against *Aspergillus niger* and *A. fumigatus*. Methanolic extract showed significant activity against *Aspergillus niger* and *A. fumigatus*. and same zone of inhibition result against *Candida, Saccharomyces* and *Microsporum* (Patil et al., 2017). The ethyl acetate and dichloromethane showed moderate zone of inhibition against fungi. The antifungal activity of n-hexane of *T. cordifolia* showed maximum zone of inhibition against *Microsporum gypseum* comparison to others. The chloroform showed constantly best zone of inhibition in all fungi among all other solvents. The standard antibiotic "Griseofulvin" showed good antifungal activity and it is considered as standard antibacterial drugs as used today which is taken as positive control against others fungal sps.

Table.2 Antifungal activity of selected solvent extracts of *T. cordifolia* (10mg/ml)

S. No.	Fungal sps.	Control	n-Hex	Chloroform	DCM	Ethyl acetate	Methanol	Aqueous
1.	*Aspergillus niger*	30mm	14mm	11mm	8 mm	13mm	16mm	18mm
2.	*Aspergillus fumigatus*	30mm	10mm	11mm	8 mm	8mm	18mm	20mm
3.	*Candida albicans*	28mm	8mm	12mm	8 mm	10mm	10mm	8mm
4.	*Saccharomyces cerevisiae*	30mm	12mm	10mm	10 mm	10mm	10mm	8mm
5.	*Microsporum gypseum*	26mm	13mm	11mm	11 mm	8mm	11mm	9mm

Control= Griseofulvin 10mg/ml

It is concluded that the antifungal activity of crude extract from the leaves of *T. cordifolia* may be due to the presence of various phytochemical constituents indulged in them (Patel.et.al. 2014). Therefore, the extracts of *T. cordifolia* could be recommended as a source of pharmaceutical and traditional drug materials required for preparation of antifungal agents (Kaur 2014). This plant different solvents extract recommended further as best antifungal drug. The antifungal activity was screened because of their great medicinal properties towards the

pathogenic organisms. The medicinal plant *Tinospora cordifolia* showed good antifungal against several organisms like *Aspergillus niger, Aspergillus fumigatus, Candida albicans, Saccharomyces cerevisiae and Microsporum gypseum* as supported by previous studies.

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