

COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF *TINOSPORA CORDIFOLIA* FRUITS EXTRACT IN DIFFERENT SOLVENTS

BIBHA KUMARI* AND MANORMA KUMARI¹

Department of Biotechnology

¹Department of Botany

A.N.College (M.U.) BODH GAYA, BIHAR

*Corresponding Author

Email:bibhakkibha@gmail.com

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ABSTRACT

The present work was carried out to evaluate the antimicrobial activity of *Tinospora cordifolia* fruits extracts in three different solvents against *Escherichia coli*, *Aspergillus niger*, and *Candida albicans*. Extracts were prepared in 70% methanol, ethyl acetate and hot distilled water separately. Antimicrobial screenings were done through well diffusion method. Through the experiments it was found that methanolic extract posses highest antimicrobial potential followed with ethyl acetate and then distilled water.

Figure : 00

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KEY WORDS : Antimicrobial activity, *Tinospora cordifolia*, Well diffusion method.

Introduction

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the rate of resistance of pathogenic microorganisms to conventionally used antimicrobial agents is increasing with an alarming frequency^{3,6,11}. In addition to this problem antibiotics are sometimes associated with adverse side effects on the host, which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppressant and allergic reactions¹. WHO has also classified antimicrobial resistance as a serious threat to every region of the world which has the potential to affect any one, of any age, in any country.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes; as a result, plants are one of the bedrocks for modern medicine to attain new principles⁴. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Further continued exploration of plant derived antimicrobials is needed today⁵.

Tinospora cordifolia belongs to the family Menispermaceae, commonly named as "Guduchi" is known for its immense application in the treatment of various diseases. *Tinospora cordifolia* is a large, deciduous, climbing shrub found throughout India. Fruits are orange-red in colour, fleshy, aggregate of 1-3 and ovoid,

smooth, drupelets on thick stalk with a sub terminal style scars. Fruits develop during winter. *T.cordifolia* is known as heart leaved Moonseed plant in English, Guduchi in Sanskrit and Giloy in Hindi. Guduchi is an Indian medicinal plant that has been used in Ayurvedic preparations for the treatment of various ailments for centuries^{2,8,10}.

Objective of this work to explore the antimicrobial potential of *T. cordifolia* fruits in three different solvents methanol, ethyl acetate and dist. water against *Escherichia coli*, *Aspergillus niger*, and *Candida albicans*.

Materials and Methods

1.Collection of plant materials:-

Fruits of *Tinospora cordifolia* were collected from the campus of A.N.College, Patna (Bihar) India.

2.Microorganism

- E.coli*
- Aspergillus niger*
- Candida albicans*

Solvent used

- Hot Distilled water
- 70% Methanol
- Ethyl acetate

Source and maintenance of Microorganism:

Pure cultures of all the experimental micro-organism were obtained from Gitanjali Patho Lab. The pure bacteria culture were maintained on nutrient Agar medium and fungus culture on P.D.A medium. Twenty-four hour old pure cultures were prepared for use each

time.

Preparation of plant extract:

Collected fruits were washed thoroughly under running tap water and then with distilled water to remove all the dust particles. Then dried into oven at 50°C. All the dried plant parts were blended in mixer grinder. The grinded powdered were sieved and stored in sterilized air tight bottle. Extraction were done through "Maceration Process". Ten g dried powdered of fruits were soaked in 100 ml solvent (distilled water, 70% methanol and ethyl acetate) separately in 250 ml sterilized conical flasks the mouth of flasks were covered with aluminium foils. Extractions were allowed to proceed for 7 days. The flask were occasionally shaken on flask shakers and warmed in water bath for better extraction. After 7 days all the plant extracts were filtered with sterilized muslin cloths in beakers separately. Then the extracts were again filtered through wattman No. 1 filter paper in a pre-sterilized flask. The mouth of the flasks were properly covered with aluminium foil and stored in refrigerator at 4°C until used. The filtered extracts were poured into pre weighted Petri plate and covered with aluminium foil with small holes and kept in an oven at 50°C for evaporation of solvent. After evaporation of the solvent the weight of petri plates were taken to know the weight of plant extract.

Weight of plant extract =

weight of petri plate with extract – weight of petri plate.

The obtained extracts were collected in small centrifuge tube at 4°C refrigerator.

Preparation of Stock Solution of Plant Extract:-

Stock solution of plant extracts were prepared by dissolving 100 mg of plant extract in 1 ml of their own solvent. The stocks were preserved at 4°C until used.

Preparation of inoculums

0.2 ml (200 µl) of overnight culture of each organism were dispensed into 20 ml of sterile nutrient both and incubated for 3-5 h to standardize the culture to 10⁶ Cfu/ml. A 100 µl of the standard culture was used for antimicrobial assay.

Culture media

Nutrient Agar and Potato Dextrose Agar were prepared according to the manufacturer's instruction, autoclaved and dispensed at 20 ml per plate in 12 x 12cm petri dishes. Set plates were incubated overnight to ensure sterility before use.

Antimicrobial Bioassay

The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measured the ability of each test sample to inhibit the *in vitro* bacterial and fungus growth. The agar well diffusion method was used in the assay^{7,9}. Wells were punched on the surface of media plates seeded with 100 µl of an overnight broth suspension of bacteria containing 10⁶ CFU/ml of organism. Different plates were prepared for each organism. Cork borer of diameter 6 mm were used to punch holes on the agar. One hundred microlitres (100 µl) each of the plant extracts at a concentration of 10 mg/ml, were introduced into the wells. The negative control used was sterile water which was employed to reconstitute the extracts, and the positive controls were 100 µg/ml Gentamicin (G) against the bacteria and 100 µg/ml Clotrimazole (C) against the fungus. The plates were allowed to put on the laboratory bench for two hour before incubating at 37°C for 24 h for bacteria and at 28°C for 48 h for fungus. The antimicrobial activity was evaluated by taking measurements of the diameters of the zones of inhibition against the test microbes.

Result and Discussion

The present study suggest that fruit extract of *T.cordifolia* showed a significant role in inhibiting the growth of tested microbes. Methanolic fruit extract showed zone of inhibition (ZOI) of 9mm, 4mm, and 3mm against *E.coli*, *Candida albicans*, and *A.niger* respectively. Ethyl acetate fruit extract showed zone of inhibition (ZOI) of 5mm, 3mm, and 2mm against *E.coli*, *Candida albicans*, and *A.niger* respectively. While hot distilled water fruit extract showed zone of inhibition (ZOI) of 3mm, 3mm,

TABLE-1 : Antimicrobial activity of *I. cordifolia* agaist solventa

Extract	Diameter of zone of inhibition (ZOI) in mm		
	Against <i>Escherichia coli</i>	Against <i>Aspergillu niger</i>	Against <i>Candida albicans</i>
Methanol	9mm	3mm	4mm
Ethyl acetate	5mm	2mm	3mm
Dist.water	3mm	—	3mm

and no result against *E.coli*, *Candida albicans*, and *A.niger* respectively (Table-1).

Thus it has been estimated that methaolic fruit extract has highest antimicrobial potential among tested

extracts, further methenolic extract was most active against *E.coli*. This difference in antimicrobial potential may be due to differences in solubility of phytochemicals in different solvents.

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