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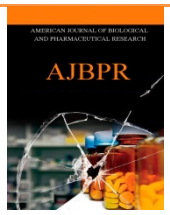

STUDY OF ANTIMICROBIAL PROPERTIES OF *TINOSPORA SINENSIS* BY AGAR WELL DIFFUSION METHOD

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ABSTRACT

Antimicrobial activity of ethanolic, methanolic, aqueous and chloroform extracts of leaves, stems and flowers of *Tinospora sinensis* were studied against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, *Candida albicans*, *Aspergillus niger* and *Aspergillus fumigatus*by agar well diffusion method. Ethanolic leaf extract of *Tinospora sinensis* had shown antimicrobial activity against *Candida albican*, *Pseudomonas aeruginosa*, and *Aspergillus niger* (with zone of inhibition 14, 13 & 8 mm respectively). Ethanolic stem and flower extract had shown antimicrobial activity against *Staphylococcus aureus* and *Aspergillus niger* (with zone of inhibition 19, 6 mm by stem extract and 8, 10 mm by flower extract). Maximum antifungal activity against *Candida albicans* was exhibited by methanolic flower extract of *Tinospora sinensis* (18 mm zone of inhibition) followed by methanolic leaf extract (13 mm zone of inhibition) and then methanolic stem extract (11mm zone of inhibition). Chloroform flower extract of *Tinospora sinensis* had shown antibacterial activity against gram negative bacteria *Pseudomonas aeruginosa* (with zone of inhibition 4 mm). Aqueous leaves stem and flower extract of *Tinospora sinensis* were not effective against any bacterial and fungal strains. Hence the ethanolic extract is more useful for preparing the antibacterial drugs while methanolic extracts are useful for antifungal extract. Present study concludes that antimicrobial activity against selected strains varies among the different plant parts used and depends largely upon the extraction procedure, type of solvent used for extraction, and the bacterial strains tested.

INTRODUCTION

India is endowed with a rich wealth of medicinal plants which have been a valuable source of natural products for maintaining human health. A large number of these medicinal plants are used in several formulations for the treatment of various diseases caused by microbes. According to World Health Organization, medicinal plants would be the source of obtaining a variety of drugs. Various societies across the world have shown great interest in curing diseases using plants/ plant based drugs. Microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. As preventive and curative measures, plants and their products are used in the treatment of infections for many centuries ago. WHO estimated that 80% of the people worldwide rely on plant based medicines for their primary healthcare [1] and India happens to be the largest user of traditional medical cure, using 7000 plant species.

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Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [2]. A wide range of medicinal plant parts are used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, many herbal industries [3]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated [4].

The increasing failure of chemotherapies and antibiotic resistance exhibited by pathogenic microbial infections agents have led to the screening of several medicinal plants for their potential antimicrobial activity [5,6]. Antibacterial properties of various plants parts, such as leaves, seeds and fruits have been well documented for some of the medicinal plants for the past two decades [7].

Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. *Pseudomonas aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections. *Staphylococcus aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditic, toxic shock syndrome (TSS), bacteraemia, and sepsis. *Candida albicans* is a causal agent of opportunistic oral and genital infections in humans. *Aspergillus niger* is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss, and, in severe cases, damage to the ear canal and tympanic membrane. *Aspergillus fumigates* causes chronic pulmonary infections or allergic disease in immune-competent hosts.

Tinospora sinensis (Syn: *Tinospora malabarica*) is a plant that grows almost throughout India and other South East Asian countries and belongs to the family Menispermaceae. The stem of this plant has great therapeutic value traditionally in treating debility, dyspepsia, fever, inflammation, syphilis, ulcer, bronchitis, jaundice, urinary disease, skin disease and liver disease [8] and known for its adaptogenic and immunomodulatory properties [9]. The aqueous and alcoholic extracts of this species are reported to have many biological potential, such as anti-inflammatory, anti-diabetic, hepatoprotective, and immunomodulatory, and adaptogenic [10]. Previous phytochemical investigations have discovered that this species contains steroids, flavonoids and alkaloids. In India, cassava is used for the treatment of ringworm, tumor, conjunctivitis, sores and abscesses. Keeping in view the importance of different type of infections caused by bacteria and fungi the present study was designed to find out antibacterial and antifungal potentiality of different plant parts of *Tinospora sinensis* against selected strains of bacteria and fungi.

stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for

MATERIALS AND METHODS

Collection of Plant Materials

The plant sample of *Tinospora sinensis* was collected from Tirumala hills, Tirupati, Chittoor district in the month of March 2012.

Procurement of microorganisms

The microorganisms were collected from Microbial type culture collection (MTCC) of Institution of Microbial Technology (IMTECH) Chandigarh and PGIMER, Chandigarh.

Extract Preparation

Leaf, flower and stem samples of *Tinospora sinensis* was thoroughly washed and dried in hot air oven at 100ºCfor 1 hr. Then its weight was noted before drying and after drying. The dried samples were then crushed in pestle and motor into fine powder.

Extract Preparation

The ethanolic and chloroform extracts of plants were prepared by following the methodology [11]. Dried powders of plants were taken and solvent was added to it in the ratio of 1:4. The aqueous extract of *Tinospora sinensis* was prepared by following the method of [12]. Dried powder was taken and distilled water was added to it in the ratio of 1:6. The methanolic extract of *Tinospora sinensis* was prepared by following the method[13]. Dried powder of Sadabaharflowers, leaves, stems were taken and methanol was added to it in the ratio of 1:10.

Culture of Test Microbes

For the cultivation of bacteria, Nutrient Agar Medium (Beef extract-1.0g, Yeast extract-2.0g, Peptone-5.0g, NaCl-5.0g, Agar-15g, distilled water-1 L) and for fungi Potato Dextrose Agar media (Potatoes infusion form 200 g, Dextrose-20.0g, Agar-15.0g, Distilled Water-1.0L final pH 5.6 at 25°C) and YEPD media (Yeast extract-3.0g, Peptone- 10.0g, Dextrose-20.0 g, Agar- 15.0g, distilled water-1.0 L) were prepared and sterilized at 15 lbs. pressure and 121℃ temperature for 25-30 min. Agar test plate, PDA plates and YEPD Plates were prepared by pouring approximately 15 ml of NAM, PDA and YEPD into the petri dish under aseptic conditions.

Agar Well Diffusion Method

The ethanol, methanol, chloroform and aqueous extracts of leaves, flowers and stems of *Tinospora sinensis* were tested by Agar Well Diffusion Method [14]. A

corkborer was sterilized by autoclaving or disinfecting it by rising in alcohol followed by sterile water. (4-mm) holes were punched aseptically in nutrient agar plate, PDA plate and YEPD Plate by using cork borer. The underside of the petri plate was marked using a wax pencil to label the wells. The cotton swabs were dipped into the broth culture of the test organisms and were gently squeezed against the inside of the tube to remove excess fluid. *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* were swabbed on Agar plates, *Candida albicans* was swabbed on PDA plates, *Aspergillus niger* and *Aspergillus fumigatus* were swabbed on YEPD plates. Swabbing was done in outside diameter of the plates. The plates were allowed to dry for about 5 minutes. . Then the extracts of *Tinospora sinensis* (30 μl) were added in 2 wells of petri plates. The ethanolic, methanolic, chloroform and aqueous solvent were used as control whereas streptomycin and penicillin were used as references for bacterial and fungal species respectively. The plates were incubated at 37ºC for 24 hrs. The zones of inhibition were measured in millimeters, using a ruler on the underside of the plate. The zone size was recorded and all the cultures were discarded in the "to be autoclaved area".

RESULTS AND DISCUSSION

From the literature survey it can be seen that *Tinospora sinensis* has been mostly studied with respect to its anticancer properties and its anti-diabetic properties. Till date, very little studies have been done on the antimicrobial properties of the plant extracts especially antifungal activity. Therefore, this study focuses on the study of antimicrobial properties of these extracts.

Ethanolic leaf extract of *Tinospora sinensis* had shown antimicrobial activity against *Candida albicans*, *Pseudomonas aeruginosa*, and *Aspergillus niger* (with zone of inhibition 14, 13 & 8mm respectively). Ethanolic stem and flower extract had shown antimicrobial activity against gram positive bacteria *Staphylococcus aureus* and fungal strain *Aspergillus niger* (with zone of inhibition 19, 6 mm respectively by stem extract and 8, 10 mm respectively by flower extract).The antimicrobial activity may be due to the presence of alkaloids. Ramya et alhad also reported variable positive result of ethanolic leaf extract of Sadbahar for different bacterial strains including both gram positive and gram negative bacteria i.e. *Escherichia coli, Pseudomonas aeruginosa, Serratiamarcescens, Salmonella typhii, Staphylococcus aureus, Streptococcus pyrogens, Bacillus cereus* and *Bacillus subtilis.*

Methanolic leaf, stem and flower extract of *Tinospora sinensis* had not shown antibacterial activity against gram positive and gram negative bacterial strains but it was effective against *Candida albicans*. Maximum antifungal activity was exhibited by methanolic flower extract of *Tinospora sinensis* (18mm zone of inhibition) followed by methanolic leaf extract (13 mm zone of inhibition) and minimum by methanolic stem extract (11 mm zone of inhibition). All plant parts in methanolic solvent had shown inhibition of *Candida albicans.* Goyal*et al.* reported that flower extract of *Tinospora sinensis* were inactive in inhibition of microbial strains (*Escherichia coli, Salmonella paratyphi, Kleibsella pneumonia, Bacillus aureus, Bacillus subtilis* and*Staphylococcus aureus*).

Table 1. Test organisms taken for antimicrobial screening

S. NO	Test organism
1.	*Escherichia coli* (MTCC No.43)
2.	*Staphylococcus aureus* (MTCC No.87)
3.	*Pseudomonas aeruginosa* (MTCC No.424)
4.	*Candida albicans* (MTCC No.1964)
5.	*Aspergillus niger* (PGIMER, Chandigarh)
6.	*Aspergillus fumigatus* (PGIMER, Chandigarh)

Fig 1. Ethanolic extract of leaves of *Tinospora sinensis*against *Candida albicans*

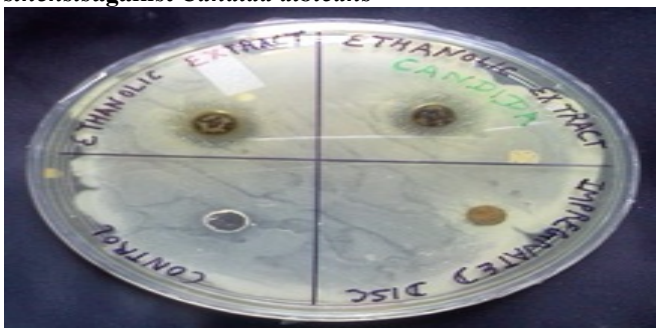
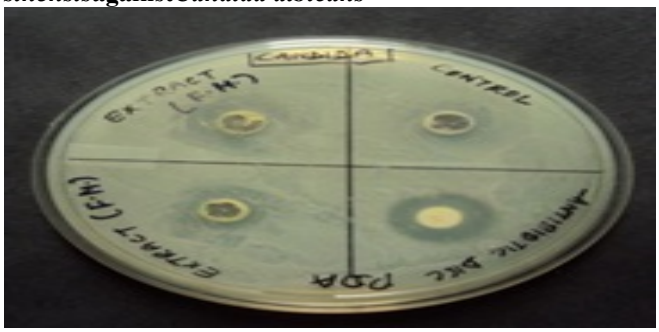


Fig 2. Methanolic extract of flower of *Tinospora sinensis*against*Candida albicans*



Aqueous leaves stem and flower extract of *Tinospora sinensis* were not effective against any bacterial and fungal strains. Only chloroform flower extract of *Tinospora sinensis* had shown antibacterial activity against gram negative bacteria *Pseudomonas aeruginosa* with only 4 mm zone of inhibition. Other chloroform plant parts extracts had not shown any antimicrobial activity. It has been shown in various studies that polarity of antibacterial compounds is crucial for their activity. Therefore it is obvious that extracts prepared using organic solvents were more active against bacterial species [15]. In a study with *Tinospora sinensis* it has been pointed out that the pattern of inhibition largely depends upon extraction procedure, plant part, physiological and morphological state of plant, extraction solvent and microorganism tested. It has been demonstrated that extracts prepared using dried plant material is much more effective than the fresh plant materials [16]. The present study concludes that the leaf extracts exhibited maximum inhibition, followed by root, stem and flower extracts. However, floral extract were comparatively inactive towards the microbial strains tested. Ethanol was found to be a more suitable solvent for the maximum extraction of active metabolites. Furthermore, Gram-positive bacteria were found more susceptible as compared to Gram-negative species. This antimicrobial study of the plant extracts demonstrated that folk medicines can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases [17].

CONCLUSION

In the present work, *in vitro* studies concluded that extracts i.e. ethanolic, methanolic, chloroform extracts except aqueous extract inhibited fungal growth and growth of gram negative and gram positive bacteria. Plant extract was ineffective against *E.coli*and *A. fumigatus.* The antimicrobial activity has been attributed to the presence of some active constituents in the extracts. This study is a substantial step and it further requires a long term study to evaluate therapeutic efficacy and toxicity of leaf, stem and flower of *C. roseus*. This result may provide a basis for the isolation of compounds from this plant. Further studies are needed to identify the pure component and establish the mechanism of action for antimicrobial activity of different parts of the plant with different extracts.

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