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Giloy (*Tinospora Cordifolia*): *In Vitro* Evaluation of Antibacterial Properties

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ABSTRACT

Antibiotic resistance has become a global concern and hence, the search for other source of antimicrobials initiated to find a way to control infections in future. The main objective of this paper is to screen Giloy (*Tinospora cordifolia*) for its antibacterial activity. The stem of *Tinospora cordifolia* is used to prepare extract for determining it's *in vitro* antibacterial activity as per the agar well diffusion method. In the agar well diffusion method 100µl of 24 hr broth culture of bacteria was aseptically and evenly swabbed on Mueller Hinton agar plates. Wells of about 8 mm diameter were aseptically cut using sterile cork-borer. 100 µl of plant extracts of different concentrations were then placed into the separate wells. The plates were incubated at 37 ºC for 24hr. Antimicrobial activity of the giloy was determined by measuring the diameter of zone of inhibition. The methanolic extract of *Tinospora cordifolia* showed 13, 11, 9 and 5 mm zone of inhibition in *S. aureus* cultures by using 100, 75, 50 and 25 mg/ml concentration, respectively while hot water extract of *Tinospora cordifolia* showed 14, 12, 10 and 8 mm zone of inhibition for *S. aureus* by using 100, 75, 50 and 25 mg/ml concentration, respectively and the cold extract of *Tinospora cordifolia* showed 10, 8, 5 and 0 mm zone of inhibition for *S. aureus* by using 100, 75, 50 and 25mg/ml concentration, respectively. The methanolic extract of *Tinospora cordifolia* indicated 12, 10, 6 and 4 mm zone of inhibition in cultures of *E.coli* by using 100, 75, 50 and 25 mg/ml concentration, respectively and the hot water extract of *Tinospora cordifolia* showed 16, 14, 12 and 10 mm zone of inhibition in cultures of *E.coli* by using 100, 75, 50 and 25mg/ml concentration, respectively. The cold water extract of *Tinospora cordifolia* showed 13, 10, 8, and 5 mm zone of inhibition in cultures of *E.coli* by using 100, 75, 50 and 25 mg/ml concentration, respectively. It has been observed that *Tinospora cordifolia* showed very promising results as indicated by the zone of inhibition of bacterial culture through agar well diffusion method that varies from few mm to few cm. This study indicates the *in-vitro* antibacterial effect of Giloy which needs further validation through *in-vivo* studies.

KEYWORDS: *Tinospora cordifolia, In-vitro,* Antibacterial properties, *E.coli, Staphylococcus aureus.*

INTRODUCTION

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern (Westh *et al.*, 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow *et al.*, 2003). However, a drastic change has taken place in the causes of fatal infections, they are still a major cause of death world over. While demographic changes and drug access issues are important reasons in the developed and developing worlds, respectively, "Relentless and Dizzying Rise of Antimicrobial Resistance" has contributed in a large measure to the persistence of infections as a major cause of morbidity and mortality. Antibiotics destroy or inhibit the growth of bacteria in concentrations that are safe for the host and can be used as chemotherapeutic agents to prevent or treat bacterial infections. Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent the infection. Thus the bacteria survive and continue to multiply causing more harm. Widespread use of antibiotics promotes the spread of antibiotic resistance. Bacterial susceptibility to antibacterial agents is achieved by determining the minimum inhibitory concentration that inhibits the growth of bacteria.

Giloy *(Tinospora cordifolia)* is one of the most widely used plants in various traditional medicinal systems including "Ayurveda". The plant is used for the treatment of jaundice, rheumatism, urinary disorder, skin diseases, diabetes, immunodeficiency and anemia. The phytochemicals present in giloy are alkaloids, diterpenoids lactones, glycosides, steroids, phenol, aliphatic compounds and polysaccharides. *Tinospora cordifolia* belongs to the family *Menispermaceae* known as Amrita or Guduchi is widely used by folk Aurvedic system and Rasayanas to improve the immune system and body resistance against infections. It is widely used in veterinary folk medicine and ayurvedic system of medicine for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and antidiabetic properties (Nadkarni and Nadkarni, 1976; Chopra *et al.*, 1958; Chopra *et al.*, 1982; Zhao *et al.*, 1991).The present study was designed to evaluate the antimicrobial activity of Giloy Satva (Extracts) using the agar well diffusion method.

MATERIALS AND METHODS

The leaves and stem of *Tinospora cordifolia* grown in the gamla without using any chemical fertiliser and/ or pesticide were collected and were washed under running tap water, air dried, cut into pieces and grinded into fine powder and stored in airtight bottles. 5gms of powdered material was extracted by cold and hot maceration method successively with 150 ml of distilled water for 2 days in sterile conical flask. The extracts were filtered using Whatman filter paper No1. The filtrates were then evaporated at 40˚C and stored in air tight bottles. For solvent extraction, 5gm of powdered material was taken in the solvents viz. hexane, acetone, chloroform, methanol and ethanol using the Soxhlet apparatus at a temperature of 30 to 35˚C. The filtrates were then evaporated at 40˚C and stored in separate air tight bottles. The plant extracts were prepared in different concentration of 100mg / ml, 75mg / ml, 50mg / ml and 25mg / ml in DMSO. The bacterial cultures of *Staphylococcus aureus* and *E. coli* were used for antibacterial activity. These bacterial strains were obtained from Institute of Microbial Technology, Chandigarh and were maintained by culturing them in nutrient agar weekly. A single colony from pure growth of test organism was transferred to 5 ml of Mueller-Hinton broth. The broth was incubated at 37ºC for 24 hours. The standardized inoculum suspension was inoculated within 15-20 minutes. The antimicrobial assay of the extracts were tested on microbial strain by agar gel diffusion method by Kirby-Bauer. In the agar well diffusion method 100µl of 24 hr broth culture of bacteria was aseptically and evenly swabbed on Mueller Hinton agar. Wells of about 8mm diameter were aseptically cut using sterile cork borer. 100 µl of extracts of different concentration were then placed into the well. The plates were incubated at 37ºC for 24hr. Microbial growth was determined by measuring the diameter of zone of inhibition (Rathore and Chauhan, 2008).

RESULTS

The results of antibacterial property of *Tinospora cordifolia* measured as millimeter of zone of inhibition is given in table 1. This study shows that the plants used in traditional medicine in India have potent antibacterial activity. Antibacterial activities were observed using the agar well diffusion technique against both type of the bacteria gram-positive *Staphylococcus aureus* and gram-negative bacteria, *Escherichia coli*. The sensitivity test was carried out with positive control i.e. ciprofloxacin. The zone of inhibition were ranges observed in different concentration of 100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml of the giloy extracts. The methanolic extract of *Tinospora cordifolia* showed 13, 11, 9 and 5 mm zone of inhibition in *S. aureus* cultures by using 100 mg/ml, 75 mg/ml 50 mg/ml and 25 mg/ml concentration, respectively. While hot water extract of *Tinospora cordifolia* showed 14, 12, 10 and 8 mm zone of inhibition for *S. aureus* by using 100 mg/ml, 75 mg/ml 50 mg/ml and 25 mg/ml concentration, respectively and the cold extract of *Tinospora cordifolia* showed 10, 8, 5 and 0 mm zone of inhibition for *S. aureus* by using 100 mg/ml, 75 mg/ml 50 mg/ml and 25mg/ml concentration, respectively. The methanolic extract of *Tinospora cordifolia* indicated 12, 10, 6 and 4 mm zone of inhibition in cultures of *E.coli* by using 100 mg/ml , 75 mg/ml, 50 mg/ml and 25 mg/ml concentration, respectively and the hot water extract of *Tinospora cordifolia* showed 16, 14, 12 and 10 mm zone of inhibition in cultures of *E.coli* by using 100 mg/ml , 75 mg/ml, 50 mg/ml and 25mg/ml concentration, respectively. The cold water extract of *Tinospora cordifolia* showed 13, 10, 8, and 5 mm zone of inhibition in cultures of *E.coli* by using 100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml concentration, respectively.

DISCUSSION

The antibiotics, 20th century wonder drug, were first developed in 1935 and thereafter, many antibiotics came in the market to treat, prevent and control the bacterial and viral diseases. However, with the passage of time and following natural law of survival of the fittest, many bacteria developed resistance against these antibiotics and situation became so worrisome that at present many superbugs are present in the environment on which presently available antibiotic drugs are not having any effect (Chauhan and Rana 2010). There has been an increase in demand for the Phytopharmaceutical products of Ayurveda in Western countries, due to the fact that the allopathic drugs might provide instant relief from the ailment but leave serious side effects. Many pharmaceutical companies are now concentrating on manufacturing of Ayurvedic Phytopharmaceutical products. There are a wide range of infectious diseases which can be cured by using medicinal plants without any side effect. The herbal formulations are easily available in the market and people can afford them as they are cheaper than the synthetic drugs which are high in cost.

In the year 1998, under the auspicious aegis of WHO, an international symposium was organized as *Eijkman Centennial on Infections in the 21st Century "Successes from the past - challenges for the future* at Hague, The Netherlands in which the main theme of discussion was that "The 20th Century wonder drug antibiotics are no more effective, then what are the alternatives to control the bacterial infections in 21st century" (Chauhan, 1998 & 1999). Thereafter, this topic was very hotly discussed at May forums of National and International conferences to find a reasonable solution. As per WHO estimate, the synthetic chemical antibiotics will become useless because of the fact of development of resistance in the bacteria and severe side effects in the host. Besides, in general there is occurrence of immunodeficiencies, either in one or another component of the immune system which also lead to the weakness in macrophage phagocytic system. Needless to mention, the present available antibiotics are of two types, bacteriostatic and bactericidal. Bacteriostatic antibiotics check the growth of bacteria and do not kill them while the remaining bacteria are to be destroyed by the macro phagocytic system. However, this system becomes weak due to the presence of several kinds of contaminants and pollutants in the food chain and is not able to cope up with the strength of the bacteria and that led to the severe bacterial infections, recurrent and persistent bacterial infections, occurrence of new diseases caused by very low pathogens or opportunistic pathogens, occurrence of severe secondary bacterial infections etc. Considering the severity and magnitude of the problem, it was thought to initiate research on the antibacterial properties of giloy (*Tinospora cordifolia*). The stem and leaves of giloy were screened for their antibacterial activity through preparation of their extracts in water, methanol etc. It has been observed that some of these plants showed very promising results as indicated by the zone of inhibition of bacterial culture through agar well diffusion method that varies from few millimeters to few centimeters which were also reported earlier by Mahesh and Satish (2008). The bacteria used in this study were common pathogen of human and animals. Besides the *in vitro* antibacterial effect of giloy, it was also known to exert its immunomodulatory effect in animals and man (Desai *et al*., 2002). Effect on enhancement of engulfment and bactericidal activity of macrophages further provide prevention and control of infectious diseases. This study indicates the *in-vitro* effect of giloy extracts which further needs confirmation and validation through *in-vivo* antibacterial effects in order to establish the antiinfection properties of giloy (Chauhan *et al*., 2003).

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Table: 1. Antibacterial sensitivity test of aquous (hot and cold) and methanolic extracts of *Tinospora cordifolia* against *Staphylococcus aureus* and *E coli* (zone of inhibition in mm)

Sr no.	**Conc. of extract**	***Staphylococcus aureus***			***Escherichia coli***		
		Hot	Cold	methanolic	Hot	Cold	methanolic
1.	100	14	10	13	16	13	12
2.	75	12	8	11	14	10	10
3.	50	10	5	9	12	8	6
4.	25	8	resistant	5	10	5	4