

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL PROPERTIES OF TINOSPORA CORDIFOLIA LEAVES EXTRACT AGAINST HUMAN BACTERIAL PATHOGENS

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ABSTRACT

Tinospora cordifolia is a large deciduous climbing shrub; the extract of the plant is used as a remedy for many diseases. In the present study the aqueous, methanol, ethanol and acetone extract of *Tinospora cordifolia*, leaves extract were screened for the presence of phytochemical components and tested for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Salmonella typhi*, *Staphylococcus epidermidis* and *Proteus vulgaris*. Results revealed the presence of anthraquinones, alkaloids, saponins, tannins, glycosides and phenolics. 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. Antibacterial activity of various extract of leaves of *Tinospora cordifolia* would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

KEYWORDS: *Tinospora cordifolia*, leaves extracts, Phytochemical Screening, antibacterial activity.

INTRODUCTION

Plants are able to produce a large number of diverse bioactive compounds. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables (Suffredini *et al.*, 2004). Plants containing beneficial

phytochemicals may supplement the needs of the human body by acting as natural antioxidants (Boots *et al.*, 2008). Various studies have shown that many plants are rich source of antioxidants. For instance, vitamins A, C, E, and phenolic compounds such as flavonoids, tannins, and lignins, found in plants, all act as antioxidants (Suffredini *et al.*, 2004). The consumption of fruits and vegetables has been linked with several health benefits, a result of medicinal properties and high nutritional value (Valko *et al.*, 2006).

Return to the nature is becoming important idea in the last decades, because of increasing side effects of drugs, drug tolerance in patients and new recombinants in genetic materials of bacteria which is responsible of drug resistance. The variation in phytochemicals compounds in different species of plants give us ability to use this material in different application, like industrial, economic and medical application (Al-Saadi, 2012). As per the reports of World Health Organization (WHO) nearly 80% of the world's population relies mainly on plant-based-traditional-medicines to meet their primary healthcare needs (WHO, 2003). It's quite interesting to note that a research paper entitled "A Neanderthal flower burial in northern Iraq" published in the renowned journal named 'Science' in the year 1975 revealed that fossil studies have confirmed the use of plants 'a means of therapy' in the Middle Paleolithic age some 60,000 years ago (Solecki and Shanidar, 1975).

Emergence of multidrug resistant pathogens has been reported to be one of the leading causes of death world 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).

Tinospora cardifolia is a large deciduous climbing shrub; 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 bark, leaves and fruits of *Tinospora cordifolia* 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 leaves of *Tinospora cordifolia*. To prove the validity of traditional medicine the present work has been undertaken to evaluate the antimicrobial screening of leaves of *Tinospora cordifolia* against the human bacterial pathogens.

MATERIALS AND METHODS

Sample Collection

Tinospora cordifolia leaves were collected from local region of Anbar City, Iraq in the month of October and authenticated by Department of Horticulture, College of Agriculture, University of Anbar, Ramadi, Anbar 31001, Iraq.

Preparation of plant material

Leaves were 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 Thirty grams of dried powder of *Tinospora cordifolia* leaves were 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

Preliminary qualitative phytochemical screening was carried out with the following methods (Khandelwal, 2001).

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 Saponins

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

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 Steroids

(Salkowski's test): Five drops of concentrated sulphuric acid (H₂SO₄) was added to 2 ml of each extract and observed for red 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 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 Anthraquinones

One gram 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. One gram 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.

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

Bacterial cultures

The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller-Hinton broth (Hi-media laboratories, Mumbai, India) at 37⁰C for 18h and then stocked at 4⁰C

in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37⁰C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10⁵ CFU/mL with the help of SPC and Nephlo-turbidometer.

Table 1: Bacterial cultures used in study.

Bacterial Pathogens	ATCC Number
<i>Proteus vulgaris</i>	426
<i>Staphylococcus epidermidis</i>	435
<i>Staphylococcus aureus</i>	96
<i>Escherichia coli</i>	739
<i>Pseudomonas aeruginosa</i>	424
<i>Klebsiella pneumoniae</i>	109
<i>Salmonella typhi</i>	733
<i>Enterobacter aerogenes</i>	111
<i>Salmonella typhimurium</i>	98

Preparation of disc for antibacterial activities

The aqueous, ethanol, methanol and acetone extracts were prepared in their respective solvents and the sterile blotting paper disc (10 mm) were soaked in the diluted extract in such concentration that the amount of solution absorbed by each disc was 1mg, 2mg, 3mg, 4mg, 5mg of each extracts of *Tinospora cordifolia* leaves. The prepared disc were dried in controlled temperature to remove excess of solvent and used in study.

Antibacterial activity using disc diffusion method

The modified paper disc diffusion method was employed to determine the antibacterial activity of aqueous, ethanol, methanol and acetone extracts. Turbidity of inoculums was matched with McFarland turbidity standard (NCCLS, 2002). Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10 mcg/disc (Hi-Media, Mumbai) were used as positive control while disc soaked in various organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18h at 37⁰C. The antibacterial activity was evaluated and diameters of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The

antibacterial activity was classified as strong (>20mm), moderate (16-19mm) and mild (12-15mm) and less than 12mm was taken as inactive.

RESULTS AND DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the exploitation of antifungal or antibacterial property of plants for developing commercial formulations for applications in crop protection. In the present study Phytochemical screening of the leaves extract of *Tinospora cordifolia* in the present study also revealed presence of terpenes and glycosides.

Table 2: Phytochemical analysis of leaves extract of *Tinospora cordifolia*.

Sr. No.	Phytochemical Constitutes	Aqueous extract	Ethanol extract	Methanol extract	Acetone Extract
1	Alkaloid	+	+	+	+
2	Flavonoids	+	++	++	+++
3	Glycosides	+	+	+	+
4	Saponins	-	++	++	+
5	Steroids	-	+	+	+
6	Tannins	+	++	+++	+++
7	Anthroquinones	-	+	+	+
8	Phenolic compounds	-	+++	+++	+++

Table 3: Antibacterial activity of *Tinospora cordifolia*, extracts against bacterial pathogens, (Zone of inhibition of growth in mm, average of 3 readings).

Medicinal Plants	Solvent extract	<i>P. vulgaris</i>	<i>S. epidermidis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>E. aerogenes</i>	<i>S. typhimurium</i>
<i>Tinospora cordifolia</i>	Aqueous	20	26	27	-	21	-	-	-
	Ethanol	22	26	25	21	21	19	17	23
	Methanol	22	32	27	22	23	17	19	18
	Acetone	22	32	27	21	21	17	19	17
Negative control	Water	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-
	Acetone	-	-	-	-	-	-	-	-
Positive control	Ampicillin (10mcg/disc)	16	25	24	11	16	18	30	19

According to antibacterial profile (Table 3), maximum inhibitory effect of the aqueous extract observed only on *Staphylococcus epidermidis*, *Staphylococcus aureus*, and moderate antibacterial against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, but mild inhibitory effect on *Salmonella typhi*, *Salmonella typhimurium*, *Proteus vulgaris*. Methanol and ethanol extract showed strong antibacterial effect against *Staphylococcus epidermidis* and *Staphylococcus aureus* and moderate antibacterial against *Proteus vulgaris*, *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhi* and *Salmonella typhimurium* but mild effect on *Pseudomonas aeruginosa*. Acetone extract showed maximum inhibitory effect on *Staphylococcus aureus*, *Proteus vulgaris*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, but moderate inhibitory effect on *Escherichia coli*, *Enterobacter aerogenes*. Several researchers have reported on the medicinal properties of plants derived compounds. These classes of compounds are known to show curative activity against several bacterial and it is not surprising that these plants extracts are used traditionally by herbalist to cure bacteria related ill-health.

Tinospora cordifolia also exerted considerable antibacterial effect against tested pathogens. However, it is ineffective against *E. faecalis* and *S. aureus* at lower concentrations with MIC value of 500 µg. This plant has been subjected to chemical investigations extensively and a number of chemical constituents belonging to different groups such as terpenoids, alkaloids, lignans and flavonoids, tannins, cardiac glycosides and steroids have been reported (Devprakash *et al.*, 2011) which may account for the antimicrobial property of this agent.

In the present study the biological activity of the acetone extract of *Tinospora cordifolia* can be attributed to the synergistic effect of the combination of flavonoids, steroids, terpenoids and saponins.

CONCLUSION

The results obtained in this study thus suggests that the identified phytochemicals may be the bioactive constituents responsible for the efficacy of leaves extract of *Tinospora cordifolia* against fever, syphilitic, ulcer, inflammatory disease wounds, conjunctivitis etc. The results of present investigation clearly indicate that the antibacterial and antifungal activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of plants, which could be of considerable interest to the development of new drugs.

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