Efficacy of *Tinospora cordifolia* against oral microflora

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Abstract

The emergence of antibiotic resistance as well as the evolution of new strains of disease causing microorganisms, are of great concern to the global health community. Effective treatment of a disease entails the formation and development of new pharmaceuticals and biomedicines. Commonly used medicinal plants could be an excellent source of drugs to prevent this problem. This study is focused on exploring the antimicrobial activity of the *Tinospora cordifolia* plant against oral microflora. The antimicrobial activity of Chloroform and Methanol extract of *Tinospora cordifolia* was screened against six cultures of oral microflora by well diffusion method. The results indicated that most of the methanol extracts exhibited antimicrobial properties. The highest potential was observed in the methanol extract of *Tinospora cordifolia* which shows the inhibition zone of 19mm against culture no 3 . The experiment confirmed the efficiency of *Tinospora cordifolia* plant extracts as natural antimicrobials and suggested the possibility of employing them in biomedicines for the treatment of oral infectious diseases caused by microorganisms.

KEYWORDS: Antimicrobial activity, *Tinospora cordifolia*, Oral microflora, Phytochemicals

Introduction

Medicinal plants have been identified for herbal remedies and healthcare product preparations. All over the world various medicinal plants have been used in daily life to cure from diseases. These plants have ability to synthesize a wide variety of phytochemical compounds that are used to protect host from attack of various microorganisms. Due to this During the antimicrobial screening of medicinal plants, priority is usually given to plant extracts who have tendency to show direct and significant inhibitory effects on microbial population Seukep etal (2019).About 14%–28% species of higher plants from about 250,000 have been scholarly investigated for the treatment of various diseases Mamedov (2012). It explains the growing interest in the field of medicinal plant biology, as about 40% of current medicines are derived from phytochemicals Gupta and Birdi (2017). Phytochemicals can be used to improve the effectiveness of the usual antibiotics after combining with them Touani (2014), Cheesman et al (2017). Plant-derived compounds are decreased potentiality of bacteria to develop resistance by interacting with main events of the pathogenic process. Thus by using these compounds in combination with antibiotics is promising approach because it allows the reuse of antibiotics that have lost their effectiveness due to multi drug resistance pumps system in bacteria Tegos etal (2002).There is huge number of

medicinal plants for example neem, aloevera ,lavender, basil, thyme,fenugreek,peppermint and sage etc.in this paper we are discussing about *Tinospora cordifolia* and its inhibitory effect against oral microflora.

Tinospora cordifolia is a medicinal plant which belongs to Kingdom: Plantae. Division: Magnoliophyta. Class: Magnoliopsida. Order: Ranunculales. Family: Menispermaceae. Genus: Tinospora and Species: T. cordifolia. *Tinospora cordifolia* is normally found in deciduous and dry forests. It is also known as Guduchi, Gllow, Tippa-teega, Shindilakodi, Amruthu, Chittamruthu,Amrutha balli, bândaul pich. Rasakinda, boraphét, geloy, guruc, gurcha, galac, garo, Amritavalli ,amrta ,cinnodbhava, Guduchi ,gulvel , Guluchi ,Gurjo etc. Kumar etal (2017)**.** it contains a huge number of phytochemical compounds such as alkaloids, steroids, terpenoids, polysaccharides, glycosides. these compounds are found almost in all parts of plant but high concentration of these compounds found mainly in the stem, leaves and roots of the plant Sinha etal (2004). Sharma etal (2012), Jamal etal (2016) reported that main compounds of *Tinospora cordifolia* are berberine , furanolactone,tinosporone, tinosporic acid, cordifolisides A-E, giloin, gilenin, crude giloininand, tinosporide, columbin, chasmanthin, palmarin, palmatosides C and F, amritosides, cordioside, tinosponone, ecdysterone, makisterone A, hydroxyecdysone, magnoflorine, tembetarine, arabinogalactan polysaccharide, picrotene, bergenin, gilosterol, tinosporol, tinosporidine, sitosterol, cordifol, heptacosanol, octacosonal, syringine, glucan polysaccharide, syringine apiosylglycoside, isocolumbin, palmatine, tetrahydropalmaitine, and jatrorrhizine.

Medicinal and therapeutic properties

The plant has been titled to many Medicinal and therapeutic properties due to these properties it is used for the treatment of urinary tract infections, gastrointestinal disorders, respiratory disease, cutaneous infections. debility, dyspepsia, fever, stomachic , diuretic, bile secretion stimulation, constipation, allays thirst, burning sensation, vomiting, jaundice and skin diseases. The root and stem of T. cordifolia are prescribed in combination with other drugs as an anti-dote to snake bite Singla and Singla (2010) Manandhar etal (2019).

Material and methods

1: Chemicals and Glassware

Borosilicate glassware was used for experimentation purpose including test tubes , flasks , petriplates . Chemicals used for the preparation of media are nutrient broth , agar powder.

2: Sample collection (Saliva samples of patients)

All the samples were collected from patients from a nearby hospital . Samples were collected in sterile cups and then transferred to sterile vials and then brought to laboratory.

3: Procurement of sample (*Tinospora cardifolia* leaves)

Tinspora cardifolia leaves were selected as sample for the antimicrobial assay and were collected from college botanical garden. After collection leaves were washed under tap water.Then they were left to dry under shade for two weeks .After the drying process ,leaves were crushed to get fine powder form and was ready to use.

4: Preparation of herb extract

10 gram of dried powder sample was mixed in 100 ml of Chloroform in a beaker and remaining 10 gram of dried powder sample was mixed in methanol containing another beaker. Both beakers were stored in sonicator for 5-6 minutes .Then the extract was filtered using whattman filter paper. The filtrate was then evaporated using water bath to get a dried extract.The dried extract was weighed and dissolved in that solvent to get a solution .Then herbal extracts were used for antimicrobial activity.

5 :Purification of Microbial Cultures

Cultures were isolated in laboratory from Oral samples of patients. Then microbial strains were purified by streaking culture on Nutrient agar plates and slants. Colonies were picked from petriplates and inoculated into the sterilized Nutrient broth .Then the flasks were incubated at 37°C for 24 hrs.

6 : Antimicrobial agent susceptibility Test

The well diffusion method was used to detect Susceptibility of *tinospora cordifolia* plant extract to antimicrobial agents on Nutrient agar . The wells were created on plates of nutrient agar with a sterile cork borer .After that cultures were inoculated by spreading inoculums over the surface of nutrient agar plates.The surface was allowed to dry for 3-5 minutes before extracts used for susceptibility tests were of different concentrations .Then plates were incubated at 37° C for 24 hours and zone of inhibition was measured.

Results

Purification of microbial strains and growth of oral microflora

Figure no. 1- Purification of microbial strains on nutrient agar plates by streaking method. a. depicted culture no. 1, b. depicted culture no. 2, c. depicted culture no. 3, d. depicted culture no.4, e. depicted culture no. 5 and f depicted culture no. 6.

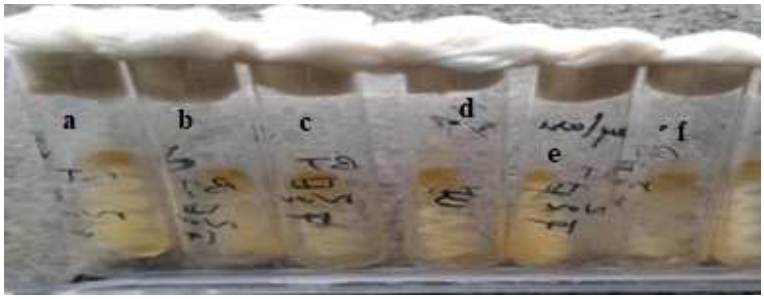

Figure no. 2-Purification of microbial strains on nutrient agar slants by streaking method. a. depicted culture no. 1, b. depicted culture no. 2, c. depicted culture no. 3, d. depicted culture no.4, e. depicted culture no. 5 and f depicted culture no. 6.

Figure no.3- Growth of oral microflora (microbial strains) in nutrient broth. a. depicted culture no. 1, b. depicted culture no. 2, c. depicted culture no. 3, d. depicted culture no.4, e. depicted culture no. 5 and f depicted culture no. 6.

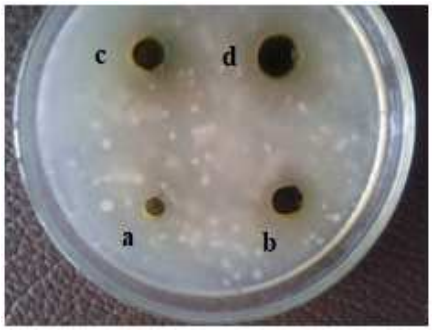

Figure 4- An inhibition zone shown by a.(25µl), b. (50µl),c. (75µl) and d. (100µl) concentration of Chloroform extract of tinospora cordifolia against oral microflora.

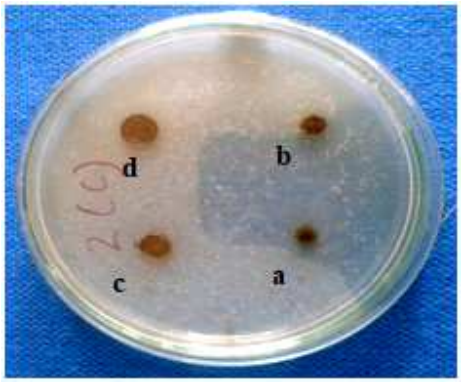

Figure no. 5- No zone of inhibition shown by a.(25µl), b. (50µl),c. (75µl) andd. (100µl) chloroform extract of tinospora cordifolia against oral microflora.

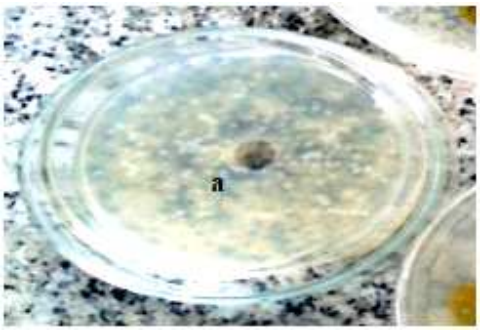

Figure no. 6-No zone of inhibition shown in case of (a) chloroform control.

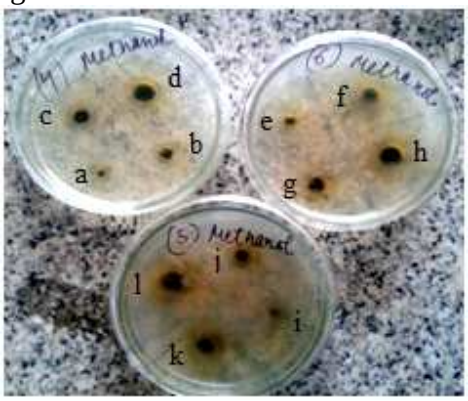

Figure no **7**. An inhibition zone shown by a.(25µl), b. (50µl),c (75µl), d. (100µl), e.(25µl), f. (50µl),g. (75µl),h (100µl), i.(25µl), j. (50µl),k. (75µl) and l. (100µl) concentration of methanol extract of tinospora cordifolia against different cultures of oral microflora.

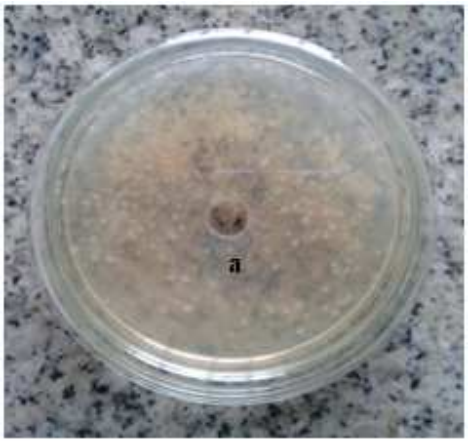

Figure no. 8- No zone of inhibition shown in case of (a) methanol control.

Effectiveness of different extracts is demonstrated by the size of the test organism growth inhibition zone around the well(diameter zone in mm).

An inhibition zone of 10 mm,11 mm,14 mm and 18mm was obtained with methanol extract of concentrations of 25 µl,50 µl,75 µl and 100 µl respectively and no inhibition zone was obtained with chloroform extract of tinospora cordifolia,methanol and chloroform control against culture no.1.

An inhibition zone of 11mm,13mm,14 mm and 16 mm was obtained with methanol extract of concentrations of 25 µl,50 µl,75µl and 100 µl and no zone of inhibition was obtained with chloroform extract, chloroform and methanol control against culture no.2.

An inhibition zone of 11mm,13 mm,15 mm and 18 mm was obtained with methanol extract of concentrations of 25 µl ,50 µl,75 µl ,100 µl and no zone of inhibition was obtained with chloroform extract, chloroform and methanol control against culture no.3.

An inhibition zone of 10 mm,15 mm,17 mm,19mm was obtained with methanol extract of concentrations of 25 µl ,50 µl ,75 µl ,100 µl respectively. An inhibition zone of –ve,9 mm,10 mm and 12mm was obtained with chloroform extract of concentrations 25 µl ,50 µl ,75 µl,100 µl respectively.no zone of inhibition was obtained with methanol and chloroform control against culture no.4.

An inhibition zone -ve,10 mm,12 mm and 15 mm was obtained with methanol extract of concentrations of 25 µl,50 µl,75 µl ,100 µl andno zone of inhibition was obtained with chloroform extract, chloroform and methanol control against culture no.5.

An inhibition zone 5mm,7mm,10 mm and 15 mm was obtained with methanol extract of concentrations of 25 µl,50 µl,75 µl ,100 µl and an inhibition zone of 10 mm,12 mm,15mm,18mm was obtained with chloroform extract of concentrations 25 µl,50 µl ,75 µl ,100 µl respectively.no zone of inhibition has been obtained with methanol and chloroform control against culture no.6.

Discussion

The enormous heritage of vast natural, time to time tested medicinal resources such as plants,veins and shrubs etc are worth exploring the possibility of developing new biomedicines which are efficient, economically viable, and clinically acceptable for human application. T. cordifolia is the one of them which is an indispensable medicinal plant also known as guduchi,giloy,amruthabali,Amruth or the “Nectar of Immortality” (in ayurveda) in recognition of its ability to impart youthfulness, vitality, and longevity. Preclinical and clinical pharmacological studies affirm the importance of its therapeutic efficacy and due to this it is used as the primary drug in the treatment of different ailments Neeraja and Margaret (2013).Earlier studies on phytochemical analysis of T. cordifolia revealed that leaf extracts of plant has flavonoids, alkaloids, phenols, tannins, steroids,terpenoids Yamaguchi et al (1998) carbohydrates,diterpines,amino acids,protein and saponin Garg and Garg (2018) due to this it is a potential medicinal plant Yamaguchi et al (1998). Presence of phytochemicals and secondary metabolites in Tinospora cardifolia are responsible for its activity against a huge number of microorganisms such as *Streptococcus mutants*, Nimri et al (1999) ,Mishra et al (2014) *Staphylococcus aureus (MTCC No.87),Proteus vulgaris (MTCC No.742), Pseudomonas aeruginosa (MTCC No.424), Bacillus subtilis (MTCC No.441), Staphylococcus epidermidis (MTCC*

No.9041), and Micrococcus luteus (MTCC No.106) Mishra *et al*.,(2014) etc. According to present study, T. cordifolia is highly antioxidant plant because of the presence of flavonoid Component (Garg and Garg 2018) flavpnoids and quinones are bind to cell wall and inactivate bacterial enzymes wherease terpenoids, polyphenols, and tannins ficilitates membrane disruption and form metal ion complexes which leads to inactivation of bacteria Choudhry et al (2013).Results obtained from our recent experiments revealed that in case of methanol extract maximum zone of inhibition was observed 19mm at concentration 100 µl and in case of chloroform extract maximum zone of inhibition was observed 18mm at concentration 100 µl against oral microflora. No zone of inhibition was observed in case of control. The observed results were similar to the results reported by Vermani etal in (2013) which stated that petroleum ether,chloroform,methanol and aqueous plant extract of *Tinospora cordifolia* showed good zone of inhibition against dental pathogens and also results reported by Samy and Ignaacimuthu in (2000) which revealed that tinospora cardifolia leaves extracts (hexane,dichloromethane,ethyl acetate,diethyl ether and methanol) are also effective against *B.subtilis,E.coli,P. vulgaris,and P mirobilis.*

Conclusion

On the basis of results this study emphasizes that leaves of tinospora cordifolia shows antimicrobial activity against oral microflora. This study supports the traditional use of *tinospora cordifolia* to cure from diseases with lesser side effects and indicated that it contains a number of phytochemicals which are responcible for its antimicrobial activity thereby proving very effective source of derived drugs.

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Tables

Table 1 Shows the antimicrobial activity of herbal extract against culture 1

Extracts	Extract conc. (25 µl)	Extract conc. (50 µl)	Extract conc. (75 µl)	Extract conc. (100 µl)	Control (100 µl)
Methanol extract (Diameter of zone) (mm)	10mm	11mm	14mm	18mm	_
Chloroform extract (Diameter of zone) (mm)	-ve	-ve	-ve	-ve	_

Table 2 Shows the antimicrobial activity of herbal extract against culture 2

Extracts	Extract conc. (25 µl)	Extract conc. (50 µl)	Extract conc. (75 µl)	Extract conc. (100 µl)	Control (100 µl)
Methanol extract (Diameter of zone) (mm)	11mm	13mm	14mm	16mm	_
Chloroform extract (Diameter of zone) (mm)	-ve	-ve	-ve	-ve	_

Table 3 Shows the antimicrobial activity of herbal extract against culture 3

Extracts	Extract conc. (25 µl)	Extract conc. (50 µl)	Extract conc. (75 µl)	Extract conc. (100 µl)	Control (100 µl)
Methanol extract (Diameter of zone) (mm)	11mm	13mm	15mm	18mm	_
Chloroform extract (Diameter of zone) (mm)	-ve	-ve	-ve	-ve	_

Table 4 Shows the antimicrobial activity of herbal extract against culture 4

Extracts	Extract conc. (25 µl)	Extract conc. (50 µl)	Extract conc. (75 µl)	Extract conc. (100 µl)	Control (100 µl)
Methanol extract (Diameter of zone) (mm)	10mm	15mm	17mm	19mm	_
Chloroform extract (Diameter of zone) (mm)	-ve	9mm	10mm	12mm	_

Table 5 Shows the antimicrobial activity of herbal extract against culture 5

Extracts	Extract conc. (25 µl)	Extract conc. (50 µl)	Extract conc. (75 µl)	Extract conc. (100 µl)	Control (100 µl)
Methanol extract +(Diameter of zone) (mm)	-ve	10mm	12mm	15mm	_
Chloroform extract (Diameter of zone) (mm)	-ve	-ve	-ve	-ve	_

Table 6 Shows the antimicrobial activity of herbal extract against culture 6

Extracts	Extract conc. (25 µl)	Extract conc. (50 µl)	Extract conc. (75 µl)	Extract conc. (100 µl)	Control (100 µl)
Methanol extract (Diameter of zone) (mm)	5mm	7mm	10mm	15mm	_
Chloroform extract (Diameter of zone) (mm)	10mm	12mm	15mm	18mm	_