



# COMPARISON OF INVITRO ANTIOXIDANT AND ANTI MICROBIAL ACTIVITIES OF ETHANOLIC EXTRACT OF TINOSPORA CORDIFOLIA AND TINOSPORA CRISPA

Mohamed Shiek Arabath S A<sup>1</sup>, Sundarapandian M<sup>2</sup>, Karthick B B<sup>3</sup>, Karthik Kumar G<sup>4</sup>, Kanaga Lakshmi B<sup>5</sup>.

Associate Professor<sup>1</sup>, Professor & HOD<sup>2</sup>

Department of Pharmacognosy<sup>1</sup>

K.M.College of Pharmacy

Madurai – Tamilnadu

India.

## ABSTRACT

Herbal medicines are complex compounds with multiple synergistic mechanisms of actions that modulate physiological functions[1]. *Tinospora* species of plant are climbing shrubs which belongs to family Menispermaceae, family. About 60 species are distributed in tropical and subtropical regions of world. Meaning of word 'Guduchi' in sanskrit is 'one which protects body against diseases'. It is designated as Rasayana in Ayurveda recommended as immunomodulator [2]. In this study the ethanolic extract of stem part of *Tinospora cordifolia* and *Tinospora crispa* were obtained by using ultrasonicator. Then the extracts have been subject to phytochemical screening followed by an In vitro antioxidant assay by DPPH method and In vitro antimicrobial assay by agar disc diffusion method. The result of the two species has been compared.

**Key words:** *Tinospora cordifolia*, *Tinospora crispa*, Antioxidant, Antimicrobial, Comparison

## 1.INTRODUCTION

*Tinospora cordifolia* is mentioned in ayurveda as rasayana to increase the life span. It possessed various pharmacological activities. Recently the Ministry of AYUSH released a notification regarding the safety of *Tinopora cordifolia* over *Tinospora crispa*, which trigger us to carry on this project. Here we would like to compare two different species of *Tinospora*. Articles review on morphological, phytochemicals and pharmacological aspects of *Tinospora cordifolia*. Leaves of *Tinospora cordifolia* shows maximum concentrated of sugar, starch, flavonoids, phenolic and tannin content as compare to aerial roots and stem. Tinosporaside and berberine were evaluated in as biomaker for cordifolia using TLC finger printing [3].

Anti-oxidant means “against oxidation”. Any substance at low concentrations compared to that of an oxidizable substrate that significantly delays or prevents oxidation of that substrate is called as Anti-oxidant. Anti-oxidant plays vital role in preserving the quality of food and maintaining health of human being [4].

The agar disc diffusion method is based on the principle that antibiotic-impregnated disk, placed on agar previously inoculated with the test bacterium, pick-up moisture and the antibiotic diffuse radically outward through the agar medium producing an antibiotic concentration gradient. A clear zone or ring is formed around an antibiotic disk after incubation if the agent inhibits bacterial growth [5]. Secondary metabolites about 65 compounds have been isolated and identified as furanoditerpenes, lactones, steroids, flavonoids, lignans and alkaloids; among these clerodane type furanoditerpenes are the characteristic compound of *Tinospora crispa* [6]. The anti-oxidant properties of *Tinospora crispa* stem distilled water extract make it useful for the treatment of disease resulting from oxidative stress such as cardiovascular disease, cancer, liver damage and renal disease [7]. The anti-oxidant properties of *Tinospora cordifolia* roots, an indigenous plant used in ayurvedic medicine in india in alloxan diabetic rats [8].

## 2. MATERIALS AND METHODS

### 2.1 COLLECTION AND AUTHENTICATION PLANT

These plants *Tinospora cordifolia* and *Tinospora crispa* were collected from Wholesale plant nursery in the panachery, Kerala and identified by Prof. Dr. Stephen Ph.D, Taxonomist, Department of Botany, American College, Madurai.

### 2.2 PREPARATION OF PLANT EXTRACT

Collected plant material washed under running tap water to remove foreign earthy adherable matter. These plants materials are cut into pieces and dried under sun for two weeks time. These plants material were crushed into fine powder by using mechanical blender and kept in an air tight polythene bags for further use and stored at room temperature. Here Ultra sonication process is adapted as extraction method. Ethanol is used as a solvent.

### 2.3 INVIRO ANTIOXIDANT ASSAY

The complexity of *in vitro* Anti-oxidant methods through several mechanisms of actions makes the comparison of each anti-oxidant method impossible. However, the *in vitro* anti-oxidant approach can provide a measurement of the effectiveness of compounds. Generally, the assessment of *in vitro* anti-oxidant activity can be divided into two main mechanisms. The first category is assessment in relation to free radicals transfer. The second category is related to the evaluation of the damaging effect on biological markers and substrates, which is based on lipid peroxidation[9].

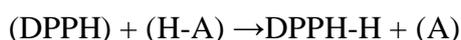
#### Procedure

##### DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al. (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

#### Principle

1, 1 Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an anti-oxidant (H-A) can be written as,



Anti-oxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the anti-oxidant compounds or extracts in terms of hydrogen donating ability.

### Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

### Working procedure

1. Anti-oxidant scavenging activity was studied using 1,1-diphenyl-2-picryl hydrazyl free radical (DPPH) (Blois, 1958) with slight modification.
2. A series of test tubes labeled from 1- 5, was taken. To each tube a concentration of 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 microgram of test solution (compounds) was added to 2.0 ml of 0.004% solution of DPPH in methanol.
3. 1ml of methanol and 2 ml of 0.004% DPPH solution was used as experimental negative control.
4. After 30 min of incubation at room temperature, the reduction in the number of free radicals was measured by reading the absorbance at 517 nm.
5. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. The percent inhibition was calculated from the following equation.
6. The standard (positive control ) used here was Quercetin.

## 2.4 ANTIMICROBIAL ASSAY

### Agar Disk-Diffusion Method

Agar disk-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the clinical and laboratory standards institute (CLSI) for bacteria and yeasts testing. Although not all fastidious bacteria can be tested accurately by this method, the standardization has been made to test certain fastidious bacterial pathogens like streptococci, Haemophilus influenza, using specific culture media, various incubation conditions and interpretive criteria for inhibition zones.

In this well known procedure, agar plates are inoculated with a standardized inoculum of the test micro-organism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The petri dishes are incubated under suitable conditions. Generally, anti-microbial agents diffuse into the agar and inhibit germination and growth of the test micro-organism and then the diameters of inhibition growth zones are measured.

Disk diffusion assay offers many advantages over other methods: simplicity, low cost, the ability to test enormous numbers of micro-organisms and anti-microbial agents, and the ease to interpret results provided [10].

## 3. RESULTS AND DISCUSSION

### 3.1 EXTRACTION

The percentage yield of ethanolic extract of *Tinospora cordifolia* (T1) is 3.55% w/w

The percentage yield of ethanolic extract of *Tinospora crispa* (T2) is 2.65% w/w

### 3.2 PHYTOCHEMICAL SCREENING

TABLE 1: Phytochemical screening of *Tinospora cordifolia* and *Tinospora crispa*

| Constituents                 | T1 | T2 |
|------------------------------|----|----|
| Alkaloids                    | +  | +  |
|                              | +  | +  |
| Flavonoids                   | -  | -  |
| Saponins                     | -  | -  |
|                              | -  | -  |
| Glycosides                   | +  | +  |
| Triterpenoids                | -  | -  |
| Tannins & Phenolic compounds | -  | -  |
| Proteins                     | -  | -  |
| Carbohydrates                | +  | +  |
| Steroids                     | +  | +  |
| Starch                       | +  | +  |

### 3.3 INVITRO ANTI-OXIDANT ASSAY

TABLE 2: Antioxidant assay by DPPH method

| Concentration<br>(microgram/ml) | % of activity(inhibition) |      |      |
|---------------------------------|---------------------------|------|------|
|                                 | Quercetin                 | T1   | T2   |
| 0.1                             | 15                        | 7.5  | 5.4  |
| 0.2                             | 24                        | 14   | 9    |
| 0.3                             | 52                        | 25   | 20.2 |
| 0.5                             | 70                        | 54.2 | 44.2 |
| 1.0                             | 88                        | 66   | 54.5 |

### 3.4 INVITRO ANTI-MICROBIAL ACTIVITY



Fig 1 – Control and Zone of inhibition of Standard & T1(25µg/ml)



Fig 2 – Control and Zone of inhibition of Standard & T1 (50µg/ml)



Fig 3 – Control and Zone of inhibition of Standard & T1 (75µg/ml)



Fig 4 – Control and Zone of inhibition of Standard & T1 (100µg/ml)

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Fig 5 – Control and Zone of inhibition of Standard & T2 (25µg/ml)

Fig 6 – Control and Zone of inhibition of Standard & T2 (50µg/ml)



Fig 7 – Control and Zone of inhibition of Standard & T2 (75µg/ml)

Fig 8 – Control and Zone of inhibition of Standard & T2 (100 µg/ml)

TABLE 3: Zone Of Inhibition Of Amikacin,T1 And T2 Vs Staphylococcus Aureus

| DILUTION (T <sub>1</sub> ) | STANDARD DISC | T1 - ZONE DIAMETER | T2 - ZONE DIAMETER |
|----------------------------|---------------|--------------------|--------------------|
| 25 µg/ml                   | AK-19mm       | 10 mm              | 9 mm               |
| 50 µg/ml                   | AK-19mm       | 12 mm              | 10 mm              |
| 75 µg/ml                   | AK-19mm       | 14 mm              | 12 mm              |
| 100 µg/ml                  | AK-19mm       | 16 mm              | 14 mm              |

The anti-microbial activity of Ethanolic Stem Extract of *Tinospora cordifolia* (25,50,75 and 100 µg/ml) and Ethanolic Stem Extract of *Tinospora crispa* (25,50,75 and 100 µg/ml) was evaluated for the potential Anti-microbial effect against *Staphylococcus aureus*.

The anti-microbial activity was determined in the extract by using Agar Disc Diffusion Method. The Zone of inhibition of different concentrations of both extracts was compared with the standard drug Amikacin for anti-microbial activity.

#### 4. CONCLUSION

It is logical to review and research in age old medicinal herbs. Guduchi is one among them which was classified under Rasayana in Ayurveda. In this study we made an attempt to compare two species of *Tinospora* that is *Tinospora cordifolia* and *Tinospora crispa*.

DPPH method is one of the established method to determine the anti-oxidant potential of the drugs. The *Tinospora cordifolia* showed 66% and *Tinospora crispa* showed 54% of anti-oxidant activity at 1000ppm and it may act as good anti-oxidant candidate. The experiment was compared with quercetin as standard.

The research shows a remarkable inhibition of the bacterial growth was shown against the tested organism. The Ethanolic Stem Extract of *Tinospora cordifolia* shows more anti-bacterial activity compare with Ethanolic Stem Extract of *Tinospora crispa*.

We would like to conclude that the present study discussed about the Phytochemical and pharmacological actions of the two species of *Tinospora* that is *Tinospora cordifolia* and *Tinospora crispa*. The well known *Tinospora cordifolia* may be more valuable crude drug than the *Tinospora crispa*.

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