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Assessment of Antioxidant and Anti - Lipid Peroxidation Capability of Guduchi (*Tinospora cordifolia*).

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ABSTRACT

Many oxidative stress related diseases like cancer, diabetes, neurodegenerative disorders and cardiovascular diseases are occurring as a result of accumulation of free radicals in the body. *Tinospora cordifolia* (Guduchi) is a medicinal herb used in the Indian system of medicine due to its health benefits. In the present study, the antioxidant activity of Guduchi extract was investigated using various *in vitro* methods such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), nitric oxide (NO), hydroxyl (OH), hydrogen peroxide radical scavenging and anti-lipid peroxidation assays. The results indicated that IC_{50} values of Guduchi extract were 0.997mg/ml in DPPH, 0.72mg/ml in ABTS, 10.7mg/ml in NO, 3.1mg/ml in H_2O_2, 0.86mg/ml in hydroxyl radical scavenging and 109mg/ml in anti-lipid peroxidation. The results revealed that Guduchi extract has promising antioxidant activity and could serve as potential source of natural antioxidants.

Keywords: Guduchi, Antioxidant, Anti lipid peroxidation, Oxidative stress.

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INTRODUCTION

The phytochemicals like flavonoids, alkaloids, tannins, carotenoids, phenolic compounds contributes to anti-oxidative qualities. In modern western medicine balance between antioxidation and oxidation is considered as a critical concept in maintaining a healthy biological system. Many traditional Chinese medicines rely on the same concept. Polyphenolic compounds are very important antioxidants due to their hydroxyl groups that confer free radical scavenging ability to plant products [1]. Numerous researches have revealed that some phenolic compounds have anticarcinogenic or antimutagenic activities, and these bioactivities of phenolic compounds might be related to their antioxidant properties [2]. Higher plants used extensively in traditional medicines are increasingly being screened for their role in modulating the activity of environmental genotoxicants. Considerable advancements were made in the natural products endowed with antimutagenic and anticarcinogenic properties. Many natural products referred to as dietary chemo-preventive compounds offer a great potential in the fight against cancer through a different range of mechanisms including antioxidant, antimutagenic activity, enzyme modulation, gene expression, apoptosis etc [3].

T. cordifolia, which is known by the common name Guduchi, is an herbaceous vine of the family Menispermaceae indigenous to the tropical areas of India, Myanmar and Sri Lanka. The plant is a climbing shrub found throughout India, typically growing in deciduous and dry forests. A variety of constituents have been isolated from *T.cordifolia* plant and their structures were elucidated. They belong to different classes such as alkaloids, diterpenoids lactones, glycosides, steroids, sesquiterpenoid, phenolic, aliphatic compounds and polysaccharides. Leaves of this plant are rich in protein (11.2%) and are fairly rich in calcium and phosphorus. The active adaptogenic constituents are diterpene compounds including tinosporone, tinosporic acid, cordifolisides A to E, syringen, the yellow alkaloid, berberine, giloin, crude giloininand, a glucosidal bitter principle as well as polysaccharides, including arabinogalactan polysaccharide (TSP). The active principles of *T.cordifolia* were found to possess complementary and immunomodulatory activities. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents. The protective effect of plant products are due to the presence of several components such as enzymes, proteins, vitamins, carotenoids, flavonoids and phenolic compounds. It is reported to possess anti-spasmodic, anti-inflammatory, anti- allergic, anti-diabetic and anti-oxidant properties [4]. During last two decades *T.cordifolia* has been subjected to extensive phytochemical, pharmacological and clinical investigations and many interesting findings in the areas of immunomodulation, anticancer activity, liver disorders and hypoglycemic are reported [5]. The changing lifestyle and food habits of human beings lead to the development of oxidative stress due to the accumulation of reactive oxygen species (ROS) there by oxidative damage of cellular components. As synthetic antioxidant chemicals possess harmful side effects, such chemicals can be replaced by naturally occurring antioxidants present mainly in herbal extracts. The aim of the present work is to evaluate antioxidative potential of *T.cordifolia*.

MATERIALS AND METHODS

The present study was focused on the responses evoked by *T.cordifolia* extract under the conditions of oxidative stress. The effect of *T.cordifolia* extract was assessed for their free radical scavenging activity and modulation of oxidative damage to lipids. The work was carried out in two phases. All the assays were carried out in triplicates.

Plant Material

Capsule containing 250mg extract of Guduchi was obtained from reputed herbal company (Himalaya drug company). The contents of the capsules were reconstituted in distilled water and used for various antioxidant assays.

Evaluation of Radical Scavenging effect of Guduchi extract

In phase I, radical scavenging effects of Guduchi extract was evaluated against DPPH, ABTS, hydrogen peroxide and nitric oxide radicals.

Measurement of DPPH Scavenging Activity

Proton-radical scavenging action is an important attribute of antioxidants, which is measured by DPPH radical scavenging assay. DPPH, a protonated radical has characteristic absorbance maxima at 518nm that decreases with the scavenging of the proton radical. Hydrogen-donating ability of the antioxidant molecule contributes to its free radical scavenging nature [6].

The Guduchi extract of varying concentrations (2-10mg/ml) was added to 1ml of methanolic solution of DPPH and total volume was made up to 3ml by methanol. The mixture was then allowed to react at room temperature for 30 minutes. Methanol was served as the blank and DPPH in methanol without the extract was served as the positive control [7]. The radical scavenging activity was calculated as follows:

$$\% \text{ Inhibition of DPPH} = \frac{A_{518}(\text{control}) - A_{518}(\text{sample})}{A_{518}(\text{control})} \times 100$$

Measurement of ABTS Scavenging Activity

The antioxidant effect of Guduchi extract was studied using ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation. ABTS•+ radicals was produced by reacting 7mM ABTS in H_2O with 2.4mM potassium persulfate stored in dark for 12hours at room temperature. The ABTS•+ solution was diluted to give an absorbance of 0.75 ± 0.05 at 745 nm in 0.1 M sodium phosphate buffer (pH 7.4). Then, 2.5mL of ABTS•+ solution was added to 1ml of Guduchi extract in varying concentrations (2-10mg/ml and 0.2-1mg/ml). The absorbance was recorded at 745nm after 10 min incubation of the mixing and percentage of radical scavenging were calculated for each concentration relative to a blank containing no scavenger. The extent of decolorization was calculated as percentage reduction of absorbance [8]. The scavenging capability of test compounds was calculated by the following equation:

$$\% \text{ Scavenging activity} = \frac{A_{745}(\text{control}) - A_{745}(\text{sample})}{A_{745}(\text{control})} \times 100$$

Measurement of Nitric oxide Scavenging Activity

Sodium nitroprusside in aqueous solution, at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that are to be estimated spectrophotometrically at 546 nm. The reaction was initiated by adding Guduchi extract of varying concentrations (2-10mg/ml) which was made upto 1ml with PBS (pH 7.4) to 2ml of sodium nitroprusside and was incubated at 37°C for 150minutes. Griess reagent (1ml) was added to 1ml of the mixture and incubated for another 30 minutes at room temperature. The absorbance was read at 546 nm against the reagent blank, in a spectrophotometer [9]. The scavenging capability of test compounds was calculated by the following equation:

$$\% \text{ Scavenging activity} = \frac{A_{546}(\text{control}) - A_{546}(\text{sample})}{A_{546}(\text{control})} \times 100$$

Measurement of H_2O_2 Scavenging Activity

A solution of H_2O_2 (40mM) was prepared in phosphate buffer. Guduchi extracts at different concentrations (2-10mg/ml) was then added to H_2O_2 solution (0.6ml) and the total volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230nm in a UV spectrophotometer. A blank solution containing phosphate buffer, without H_2O_2 was prepared [10]. The extent of H_2O_2 scavenging of the plant extracts was calculated as:

$$\% \text{ Scavenging activity} = \frac{A_{230}(\text{control}) - A_{230}(\text{sample})}{A_{230}(\text{control})} \times 100$$

Oxidative damage to Biomolecules and Effect of Guduchi extract

In Phase II, the effect of Guduchi extract on the extent of oxidative damage to cellular biomolecules like lipids and DNA was evaluated.

Anti-Lipid peroxidation Assay

Preparation of goat liver slices: The goat liver was collected fresh from a slaughter house and was plunged into cold sterile PBS and maintained at 4°C till use. Thin slices of 1mm thickness were cut using a sterile scalpel.

Methodology: One gram of goat liver slice was taken in 4ml of sterile PBS in a test tube. The oxidising agent 30% H_2O_2 (200µl) and the Guduchi extract of varying concentrations (2-10mg/ml) was added and incubated at 37ºC with mild shaking for one hour. Appropriate controls were also set up. After incubation, the goat liver slices were homogenized in the incubation medium using a Teflon homogenizer and the homogenate was used for the assay. The reaction was terminated by mixing 1ml of homogenate and 2ml of TBA-TCA-HCl reagent. The contents were incubated in a boiling water bath for 15 minutes and the colour change developed was estimated at 535nm against a reagent blank, in a spectrophotometer.

$$\% \text{ Inhibition} = \frac{A_{535}(\text{control}) - A_{535}(\text{sample})}{A_{535}(\text{control})} \times 100$$

Deoxyribose Assay (Hydroxyl radical scavenging)

Deoxyribose is oxidized when exposed to hydroxyl radicals, such a degradation can be detected by heating the products in the presence of thiobarbituric acid under acidic conditions, which leads to development of a pink chromogen, and measuring absorbance at 532 nm against appropriate blank. The reaction mixture contained 100µl of 28mM 2-deoxy ribose (dissolved in 20mM phosphate buffer with pH 7.4), 100µl of 0.1mM EDTA, 100µl of 0.1mM $FeCl_3$, 100µl of 0.1mM ascorbic acid, 100µl of 1mM H_2O_2 and 100µl of different concentrations of Guduchi extract (2-10mg/ml). After an incubation period of 1hr at 37º C, 1ml of TBA and 1ml of TCA were added to all the tubes and were heated at 95º C in water bath for 20minutes. The absorbance was read at 532nm after cooling. The test was carried out in duplicates [6]. The % inhibition was calculated by the formula;

$$\% \text{ Inhibition} = \frac{A_{532}(\text{control}) - A_{532}(\text{sample})}{A_{532}(\text{control})} \times 100$$

Statistical analysis

The data were expressed as mean ± S.D. for triplicate readings. The statistical analysis was done using Microsoft Excel 2007.

RESULTS AND DISCUSSION

Evaluation of Radical Scavenging effect of Guduchi extract

The DPPH anti-oxidant assay is based on the ability of DPPH, a stable free radical to decolorize in the presence of anti-oxidants. Hasan et al., has shown the DPPH scavenging activity of Guduchi extract (GE) with an IC_{50} value of 0.02mg/ml [11]. In a study by Jain et al., the calculated IC_{50} value was 97µg/ml [**12**]. In our study GE showed significant DPPH scavenging activity at concentration range of 2-10mg/ml (Fig.1a). The GE was fast and effective scavenger of the DPPH radical when compared to other radicals because even at

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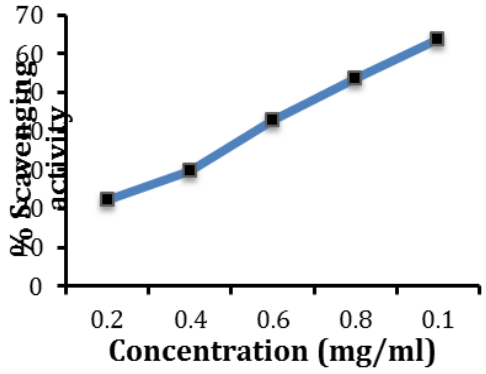
2mg/ml it showed 63% scavenging. From Fig.1a it is clearly understood that from 2-6mg/ml GE demonstrated an increasing trend of scavenging activity and then almost constant. It was observed that the extract possesses DPPH scavenging activity with IC_{50} value of 0.997mg/ml. The ABTS assay is based on the inhibition of the absorbance of radical cation ABTS+, by antioxidants that have a characteristic wavelength at 734 nm. In an early study by Jain et al., IC_{50} for ABTS scavenging activity was found to be 94µg/ml in aqueous extract and 85µg/ml in ethanolic extract of *T.cordifolia* [12]. GE showed very good response against ABTS radical at 0.2-1mg/ml. The percentage scavenging effect on the ABTS radical was increased with the increase in the concentration of GE from 0.2-1.0mg/ml (Fig. 1b). The percentage scavenging was varying from 22% in 0.2mg/ml to 63% in 1.0mg/ml of GE. It was observed that the extract possesses ABTS scavenging activity with IC_{50} value of 0.72mg/ml.



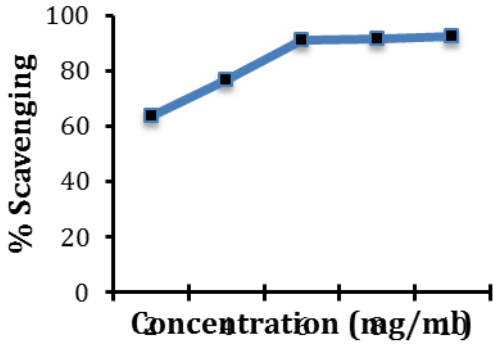
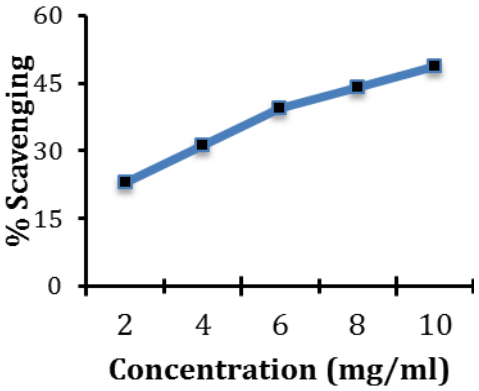


Fig. 1a **Fig. 1b**

Figure 1: (a) DPPH scavenging activity of Guduchi extract, (b) ABTS scavenging activity of Guduchi extract

In previous study by Jain et al., observed IC_{50} value of Guduchi extract for NO scavenging activity was 85µg/ml [**12**]. GE showed comparatively less response to NO scavenging activity. The percentage scavenging effect on the NO radical was slowly increasing with the increase in the concentration of GE from 2-10mg/ml (Fig.2a).



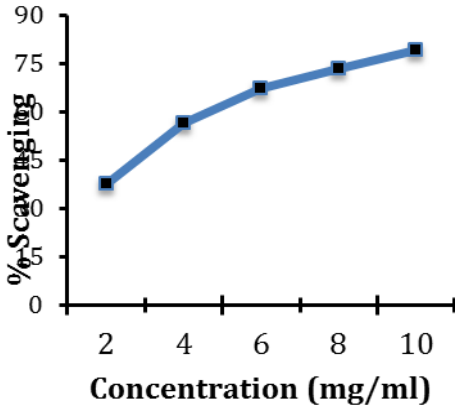


Fig.2a **Fig.2b**

Figure 2: (a) Nitric oxide radical scavenging activity of Guduchi extract, (b) Hydrogen peroxide radical scavenging activity of Guduchi extract

The percentage scavenging was varying from 22% in 2mg/ml to 48% in 10mg/ml of GE. It was observed that the extract possesses NO scavenging activity with IC_{50} value of 10.7mg/ml.

GE showed moderate response to H2O2 scavenging activity at 2-10mg/ml concentration as shown in Fig. 2b. The percentage scavenging effect on the H2O2 radical was slowly increasing with the increase in the concentration of GE from 2-10mg/ml (Fig.2b). The percentage scavenging was varying from 37% in

2mg/ml to 79% in 10mg/ml of GE. It was observed that the extract possesses H2O2 scavenging activity with IC50 value of 3.1mg/ml.

GE showed less response to anti-lipid peroxidation assay when compared to *in vitro* radical scavenging assays at 2-10mg/ml (Fig.3a). The percentage inhibition of lipid peroxidation was very less at 2mg/ml GE and was slowly increasing with the increase in the concentration upto 10mg/ml. The percentage scavenging was varying from 9% in 2mg/ml to 27% in 10mg/ml of GE. It was observed that the extract possesses percentage inhibition with IC_{50} value of 109mg/ml.

Deoxyribose is important component of DNA. GE demonstrated good response to hydroxyl radical mediated degradation of deoxyribose at 2-10mg/ml (Fig. 3b). At 2mg/ml GE showed a good percentage inhibition of 70% and from Fig. 3 b it is understood that from 2-4mg/ml GE is showing an increase in scavenging activity and then almost constant. Deoxyribose is important component of DNA. It depicts that GE can be a good DNA protectant against hydroxyl radical. The percentage scavenging was varying from 70% in 2mg/ml to 90% in 10mg/ml of GE. It was observed that the extract possesses percentage inhibition with IC_{50} value of 0.86mg/ml.

The results from various free radical scavenging systems revealed that *Tinospora cordifolia* (Guduchi extract) has good antioxidant property, with some varying scavenging activities for different reactive oxygen species (ROS) at different magnitudes of potency.

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