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# EVALUATION OF IN-VITRO ANTIOXIDANT POTENTIAL ON ETHANOLIC EXTRACT OF ROOT OF *SMILAX CHINA*

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## ABSTRACT

**Objectives:** The objective of the present work *In vitro* antioxidant activities of ethanolic extract of root of *Smilax china* was investigated.

**Materials and Methods:** The root of *Smilax china* was carried out as extraction made by various solvent system like petroleum ether, chloroform, ethyl acetate, methanol and water. The percentage yields of extracts were more and many phytoconstituents also available in the ethanolic extract of root of *Smilax china.* So the ethanlic extract was selected for this study. The free radical scavenging activity to evaluate by DPPH (diphenyl-β-picrylhydrazyl) radical scavenging activity, Hydroxyl radical scavenging activity**,** FRAPS method and estimation of total flavonoids.

**Results:** DPPH radical scavenging activity of ethanolic extract and standard rutin $IC_{50}$ values was found to be 428 µg/ml and 480 µg/ml, Hydroxyl radical scavenging activity of ethanolic extract and reference standard Ascorbate $IC_{50}$ values was found to be 245 µg/ml and 410 µg/ml. FRAP method of methanolic extract and reference standard Ascorbate $IC_{50}$ values was found to be 320 µg/ml and 50 µg/ml. The total flavonoids content of ethanolic extract was found to be 5.47mg/g . The above result possess significant antioxidant activity when compare to the above all standard.

**Conclusion:** These *in-vitro* assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

**Keywords:** *Smilax china*, DPPH assay, Hydroxyl radical scavenging activity, FRAP method, total flavonoids.

## INTRODUCTION

Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions ($O^{2-}$) and hydroxyl radicals (OH'), as well as nonfree- radical species such as hydrogen peroxide[1,2]. In living organisms various ROS can form in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionising radiation, certain pollutants, organic solvents, and pesticides [3]. Ethanomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties.

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The root of *Smilax china* is a belong to the Liliaceae family. *Smilax china* (Liliaceae) is a deciduous climber with rounded leaves and red berries. The root tubes of which furnish the drug known as china root. It is found in the south Indian states namely Andra Pradesh, Tamilnadu and Karnataka. It possesses anti-inflammatory, diuretic, anti-diabetic, anti-psoriatic, digestive properties. It is also heptoprotective, nephroprotective and used in cases of infertility. Till now various pharmacological activities had been done on different parts and extracts of the plant *Smilax china* like chronic pelvic inflammatory diseases[4] promoting blood circulation[5] inhibit AA mouse's secondary inflammatory swelling, reduce thymus and spleen weights, decrease CD4/CD8, but had little influence on B Cell. It acts regulating cell-mediated immunity, but has little effect on humoral immunity[6] anti microbial[7] chronic pelvic inflammation[8] anti-inflammatory and anti-nociceptive activities[9] anti-inflammatory effects on acute and chronic inflammation[10], inhibitory effects on

cyclooxygenase-2 enzyme (COX-2) and production of TNFalpha (tumor necrosis factor alpha) in murine peritoneal macrophages[11] anticancer activity against HeLa cells[12] chronic pelvic anti inflammatory activity[13] , *in vitro* anti microbial activity[14] anti-hyperuricemic and nephroprotective activity in hyper uricemic animals[15] hepato protective activity[16] antidiabetic activity[17] anticonvulsant and neurotoxic effects[18] spermatological activity[19] antioxidant and antimicrobial in food and cosmetic industry[20] anti-HIV -1 activity[21] anti-obesity activity[22], endothelial dysfunction study[23], and Anti-metastatic activity[24] .Therefore, the present investigation focused to evaluate the invitro antioxidant potential of ethanolic extract of root of Smilax china in different screening methods.

## MATERIALS AND METHODS

*Collection and Identification of Plant materials:*
The roots of smilax china were collected form Tirunelveli District of Tamilnadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The root of *Smilax china* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

*Preparation of Extracts:*
The above powdered plant materials were consecutively extracted with Petroleum ether (40-60$^0$C) by hot continuous percolation method in soxhlet apparatus [25] for one day. The marc was dried out and extracted with chloroform and then marc was extracted with ethyl acetate (76-78$^0$C) for one day, then this marc was dried out after that it was extracted with ethanol for one day and then marc was extracted with water. All the three extracts were concentrated by utilizing a rotary evaporator and undergone to freeze drying using a lyophilizer until dry powder was acquired. The ethanolic extract gave more yield and more phytoconstituents were present. So the ethanolic extract of *Smilax china was* selected for the further investigation.

## RESULTS

**Evaluation of antioxidant activity of *in vitro* studies:**
*DPPH photometric assay:* [26]
A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518nm and converted into percentage radical scavenging activity as follows.
Where A518 control is the absorbance of DPPH radical with methanol; A518 sample is the absorbance of DPPH radical with sample extract/ standard.

*Hydroxyl radical scavenging activity:* [27]
Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. This method involves in-vitro generation of hydroxyl radicals using Fe3+/ascorbate/EDTA/H2O2 system using Fenton reaction. Scavenging of this hydroxyl radical in presence of antioxidant is measured. In one of the methods the hydroxyl radicals formed by the oxidation is made to react with DMSO (dimethyl sulphoxide) to yield formaldehyde. Formaldehyde formed produces intense yellow colour with Nash reagent (2M ammonium acetate with 0.05m acetic acid and 0.02m acetyl acetone in distilled water). The intensity of yellow colour formed is measured at 412 nm spectrophotometrically against reagent blank. The activity is expressed as % hydroxyl radical scavenging

*FRAP Method:* [28]
FRAP (Ferric Reducing Antioxidant Power) is one of the most rapid test and very useful for routine analysis. The antioxidative activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ (2,4,6 – tri) (2-pridyl)–S-triazine) and FeCl36H2O. The absorbance was measured spectrophotometrically at 595nm. Antioxidant activity of plant extracts is reported by this method.
*Total flavonoids:* [29]
0.2g of the plant material was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and these 2 ratios were combined. This was evaporated to dryness until most of the ethanol has removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was pipette-out in a test tube. 4 ml of the vanillin reagent (1% vanillin in 70% conc. H¬¬2¬SO4¬) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

*DPPH Photometric Assay:*

The percentage of DPPH radical scavenging activity of ethanolic extract of *Smilax china was* presented in Table 1. The ethanolic extract of *Smilax china* exhibited a maximum DPPH scavenging activity of 59.45% at 1000 µg/ml whereas for Rutin(standard) was found to be 69.83% at 1000 µg/ml. The $IC_{50}$ of the ethanolic extract of *Smilax china and* rutin were found to be 428µg/ml and 480µg/m respectively.

*Hydroxy radical method:*

Free radical scavenging activity of ethanolic extract of *Smilax china* was determined by hydroxyl radical method was presented in Table 2. The free radical scavenging potential shown maximum activity is 63.89 at 1000µg/ml for as Standard (ascorbate) was found to be 62.03 at 1000 µg/ml. The $IC_{50}$ of the methanol extract of Smilax china and standard (Ascorbate) was found to be 245µg/ml and 410µg/ml respectively.

*FRAP method:*

Reduced ability of the ethanolic extract of *Smilax china was* determined by FRAP method was presented in Table 3. Maximum reduced ability of ethanolic extract of *Smilax china is* 70.54% at 1000µg/ml and Standard (Ascorbate) was found to be 98.07% at 1000 µg/ml. The $IC_{50}$ of the ethanolic extract of *Smilax china* and standard (Ascorbate) was found to be 320µg/ml and 50µg/ml in better antioxidant is respectively.

**Table-1: Antioxidant activity on root of ethanolic extract of *Smilax china* on DPPH Photometric assay.**

| S.No | Concentration (µg/ml) | % of activity (±SEM) | |
|---|---|---|---|
| | | Sample (Ethanolic extract) | Standard (Rutin) |
| 1 | 125 | 36.45±0.12 | 18.85 ± 0.076 |
| 2 | 250 | 45.29±0.92 | 22.08 ± 0.054 |
| 3 | 500 | 52.34±0.05 | 52.21 ± 0.022 |
| 4 | 1000 | 59.45±0.18 | 69.83 ± 0.014 |
| | | **$IC_{50}$=428 µg/ml** | **$IC_{50}$ = 480 µg/ml** |

*All values are expressed as mean ± SEM for three determinations

**Table-2: Antioxidant activity of root of ethanolic extract of *Smilax china* on Hydroxy radical method**

| S.No | Concentration (µg/ml) | % of activity (±SEM) | |
|---|---|---|---|
| | | Sample (Ethanolic extract) | Standard (Ascorbate) |
| 1 | 125 | 34.26±0.76 | 27.63±0.076 |
| 2 | 250 | 50.45±0.32 | 49.53 ±0.054 |
| 3 | 500 | 57.12±0.29 | 55.12±0.022 |
| 4 | 1000 | 63.89±0.82 | 62.03±0.014 |
| | | $IC_{50}$=245 µg/ml | $IC_{50}$=410µg/ml |

*All values are expressed as mean ± SEM for three determinations

**Table-3: Reducing ability of root of ethanolic extract of *Smilax china* by FRAP method**

| S.No | Concentration (µg/ml) | % of activity (±SEM) | |
|---|---|---|---|
| | | Sample (Methanolic extract) | Standard (Ascorbate) |
| 1 | 125 | 27.56±0.14 | 72.04±0.014 |
| 2 | 250 | 44.98±0.25 | 82.05±0.034 |
| 3 | 500 | 58.45±0.45 | 86.04±0.026 |
| 4 | 1000 | 70.54±0.48 | 98.07±0.041 |
| | | $IC_{50}$= 320 µg/ml | $IC_{50}$=50µg/ml |

*All values are expressed as mean ± SEM for three determinations

**Table-4: The total flavonoids content of ethanol extract of root of *Smilax china***

| S.NO | Extract | Total Phenolic content (mg/g Catechol) | Total Phenolic content ±SEM |
|---|---|---|---|
| 1 | Ethanol extract of root of *Smilax china* | 5.74<br>5.18<br>5.48 | 5.47±0.18 |

*All values are expressed as mean ± SEM for three determinations

*Total flavonoids:*

The total flavonoids content of ethanol extract of root of *Smilax china was* presented table 4. Based on the report of ethanolic extract of root of *Smilax china was* found 5.47 mg/g of total flavonoids compound.

**DISCUSSION**

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation [30]. The *in-vitro* antioxidant activities of the roots of *Smilax china* was evaluated by DPPH free radical scavenging activity, Hydrogen peroxide scavenging activity and Ferric reducing power assay were carried out. The studies were carried out taking rutin and ascorbic acid as the standard antioxidant which is also a natural antioxidant. The results of antioxidant activities were expressed in terms of % inhibition of generated free radicals respectively with respect to various concentrations. Concentration dependent activity was observed in each case i.e; higher concentrations of the extracts were found to exhibit higher % inhibition in each procedure of the antioxidant activity. With reference to the observed IC50 value of roots of *Smilax china*, the antioxidant potential was found to be highest in case of hydrogen peroxide scavenging activity and it had the following order- hydrogen peroxide Scavenging Activity (IC50 - 245 µg/ml) > ferric reducing assay (IC50 -320 µg/ml >DPPH Free Radical Scavenging Activity (IC50 -428µg/ml).The flavonoid content of the ethanolic extract of roots of *Smilax china* was found 5.47 mg/g Catechol. The flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity [31]. It is well known that flavonoids and polyphenols are natural antioxidants but have also been reported to significantly increase SOD, glutathione and catalase activities.

**CONCLUSION**

The results of the above investigation indicated that the ethanolic extract of root of *Smilax china* showed strong antioxidant activity in DPPH (diphenyl-β-picrylhydrazyl) radical scavenging activity, Hydroxyl radical scavenging activity and FRAP method. In addition, the ethanolic extract of *Smilax china* was found to contain a noticeable amount of total flavonoids, which play a major role in controlling antioxidants. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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