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IN VITRO ANTIOXIDANT ACTIVITY ON THE ROOT TUBER OF SMILAX CHINA L.

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ABSTRACT

Many medicinal plants were with a long history of use in folk medicine against a variety of diseases. Recently, many researchers have taken a great interest on medicinal plants for their phytochemical constituents and biological activities including antioxidant activity. The present study was aimed to evaluate phytochemical analysis, quantification of total phenolic, alkaloid contents and antioxidant activity of *Smilax china* extracts (70% Ethanol, Methanol, Ethyl Acetate and Hexane). The total phenolic and alkaloid contents were quantified by using gallic acid and atropine are as standards and antioxidant activity was evaluated by using three free radicals (Superoxide and DPPH). *Smilax china* extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, flavanoids, alkaloids, glycosides, tannins, carbohydrates, and amino acids. The methanol extract has more phenolic and alkaloid contents i.e. 38.83±0.68 (mg/gm) and 26.37±0.16 (mg/gm) than other extracts. The extracts were produced concentration dependent percentage inhibition of free radicals and produced maximum activity at concentrations of 800µg. Among all extracts, methanolic extract showed better activity compared to other extracts with mean IC_{50} values DPPH radicals were 800 µg and 400µg. The above results suggest that *Smilax china* extracts have antioxidant activity. Further research is in progress i.e. to isolation and characterization of active molecules (compounds) responsible for the antioxidant activity which can be used to treat various diseases.

KEY WORDS: ***Smilax*** **china, Free radicals, Antioxidant Activity, Total Alkaloids.**

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INTRODUCTION

A free radical is an atom or group of atoms that has at least one unpaired electron and is therefore unstable and highly reactive. Human beings are exposed to free radicals in the environment through Radiation and pollution. Free radicals attack and damage cell membrane, cell organelles including DNA, leading to faulty translation of genetic material [1,2] . Their action is opposed by a balanced and coordinate system of antioxidant defenses which detoxify the reactive intermediates or easily repair the resulting damage. Antioxidants scavenge these free radicals and enable cells to rejuvenate or stabilize the process of life. Upsetting this balance causes oxidative stress, which can lead to cell injury and death [3]. Reactive oxygen Species and free radicals formed during oxidation have been reported to contribute to diseases such as cancer , diabetes, cardiovascular diseases and aging [4]. Ayurveda, an ancient Indian system of medicine is rapidly gaining global acceptability as a highly effective healthcare system. Many drugs in ayurveda derived from medicinal plants have been reported as rich sources of antioxidants and th e use of such natural resources as diet supplements will help in reducing the inci dence of many diseases related to oxidative Stress [5]. The Genus Smilax L. consists of more than 300 Species, distributed all over the world, out of which 24 are found in india [6]. Four species Viz . Smilax aspera , Smilax perfoliota , Smilax china and Smilax zeylanica occur in the forests and hills of south india [7,8]. Species of *Smilax* are reported to contain phytoconstituents dioscin (spirostanol triglycoside), plant steroids such as smilagenin and sarsapogenin. The roots of *Smilax china* have a steroidal saponin glycoside diosgenin . The present work is under taken to ascertain the antioxidant potential of Smilax china. In this study invitro antioxidant activities of Smilax china china root tuber is presented.

MATERIALS AND METHODS

COLLECTION AN IDENTIFICATION OF PLANT MATERIAL

The root tubers of *Smilax china*. were collected from the vicinity of Tirunelveli District, Tamil Nadu, India, during June 2012. The plant material was identified and aut henticated by National Institute of Siddha Medicine, Chennai, and Tamilnadu, India. Prior to the extraction, the root tubers are dried and powdered and stored in air tight containers.

QUANTIFICATION OF TOTAL PHENOLIC CONTENT[9]

Total phenolic content was determined using the Folin-Ciocalteau reagent Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765nm.The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/gm. (GAE).

QUANTIFICATION OF TOTAL ALKALOID CONTENT[10]

Total alkaloid content was determined by Fazel *et al.,* method. The plant extract (1mg/ml) was dissolved in 2 N HCl and then filtered. The pH of a phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. By using standard atropine calibration curve, measured the concentration of alkaloid content in atropine

equivalents using unit's mg/gm. (GAE).

IN VITRO ANTI-OXIDANT ACTIVITY

For the assessment of free radicals scavenging activity of hexane, ethyl acetate, Ethanol (70%v/v) and methanol extracts were dissolved in dimethyl sulphoxide (DMSO) respectively.

DPHH RADICAL SCAVENGING ACTIVITY

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca [11]. In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine. Lower the absorbance higher the free radical scavenging activity [12].

RESULTS AND DISCUSSION

PHYTOCHEMICAL ANALYSIS AND QUANTUFICATION OF TOTAL PHENOLIC AND ALKALOID CONTENTS

Qualitative phytochemical screening of *Smilax* extracts revealed the presence of different phytochemical constituents like steroids, terpenoids,flavanoids,alkaloids, glycosides, tannins, carbohydrates, oils and amino acids. The extracts gave negative results for the quinines and saponins. The Quantified phenolic contents of Smilax china extracts were ranging from 13.52±0.19 to 38.83±0.68. (mg/gm). The methanol extract has more phenolic content i.e. 38.83±0.68 (mg/gm) than other extracts and the alkaloid content was ranging from 17.73±0.38 to 32.64±0.86 (mg/gm). The methanolic extract has more alkaloid content i.e. 26.37±0.16 (mg/gm) than other extracts. The results were shown in table 1.

Table 1
Total phenolic and alkaloid contents (mg/gm) of Smilax china

S.NO.	NAME OF THE EXTRACT	TOTAL PHENOLIC CONTENT (mg/gm)	Total alkaloid content (mg/gm)
1	Hexane	13.54 ± 0.19	17.73 ± 0.38
2	Ethyl acetate	27.46 ± 0.32	21.47 ± 0.25
3	Methanol	38.83 ± 0.68	32.64 ± 0.86
4	Ethanol -70%	31.58 ± 0.47	26.49 ± 0.18

IN VITRO ANTI-OXIDANT ACTIVITY

The reactive oxygen species or oxidants, which are formed in the human body due to exogenous and endogenous factors, are found to be responsible for many diseases. Antioxidants are reducing agents and limit oxidative damage to biological structures by passivating free radicals. Antioxidant compounds may function as free radical scavengers, completers of prooxidant metals, reducing agents and quenchers of singlet oxygen formation [13]. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability. Day by day, a lot of researches have shown the potential of phytochemical antioxidants as health benefactors because of their ability to neutralize free Radicals, reactive oxygen species, or oxidants responsible for the onset of cell damage.

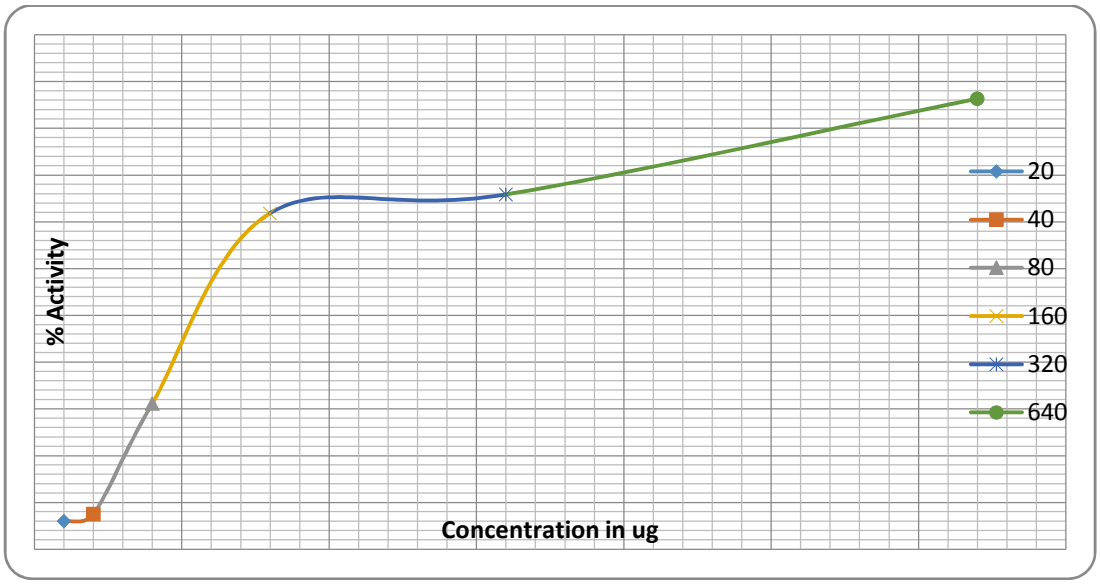
Table 2
DPHH RADICAL SCAVENGING ACTIVITY OF STANDARD (ASCORBIC ACID)

Concentration in µg/ml	Absorbance at 517nm				Activity (%)
	R_1	R_2	R_3	Average	
0 (CONTROL)	0.8832	0.8843	0.8845	0.8839	-
20	0.8308	0.8310	0.8309	0.8309	5.99
40	0.8175	0.8178	0.8182	0.8178	7.47
80	0.6086	0.6089	0.6090	0.6088	31.12
160	0.2490	0.2490	0.2490	0.2490	71.82
320	0.2133	0.2138	0.2135	0.2135	75.84
640	0.0323	0.0327	0.0325	0.0325	96.3

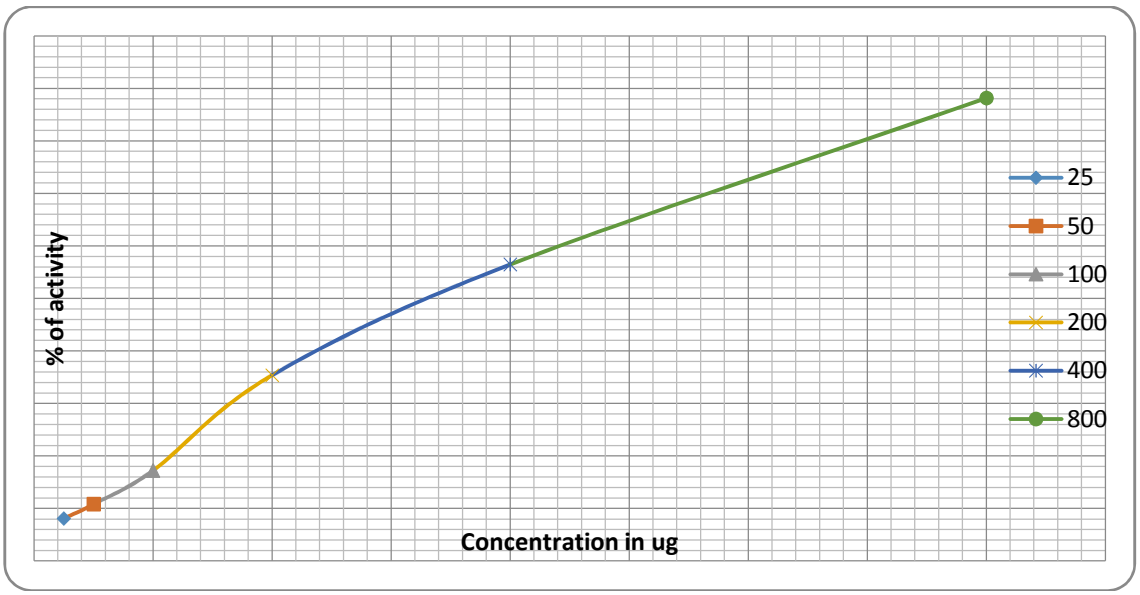
Table 3
DPHH RADICAL SCAVENING ACTIVITY OF SMILAX CHINA AT DIFFERENT CONCENTRATIONS

Concentration in µg/ml	Absorbance at 517nm				Activity (%)
	R_1	R_2	R_3	Average	
25	0.8128	0.8127	0.8129	0.8128	8.04
50	0.7886	0.7885	0.7889	0.7886	10.78
100	0.7322	0.7317	0.7317	0.7318	17.20
200	0.5710	0.5713	0.5709	0.5710	35.39
400	0.3850	0.3844	0.3851	0.3848	56.46
800	0.1051	0.1049	0.1049	0.1049	88.13

GRAPH 1
SHOWING DPHH RADICAL SCAVENGING ACTIVITY OF THE STANDARD (ASCORBIC ACID)



GRAPH 2
SHOWING DPHH RADICAL SCAVENGING ACTIVITY OF SMILAX CHINA AT DIFFERENT CONCENTRATION



INHIBITION OF DPHH ACTIVITY

The DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radicals was initiated by the lipid autoxidation. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [14]. The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm, which is induced by anti-oxidants. Positive DPPH test suggests that the samples were free radical scavengers. The scavenging effect of *Smilax china extracts* and ascorbic acid on DPPH radical was compared. On the DPPH radical, *Smilax china extracts* had scavenging effects with increasing concentration in the range of 200- 800 µg/ml. when compared with ascorbic acid; the scavenging effect of Smilax china *extracts* was lower. The IC50 values of Hydro-alcoholic, methanol, ethyl acetate and hexane extracts and ascorbic acid were found to be 226µg, 313µg, 377µg, 546µg and 16 µg respectively.

CONCLUSION

The scavenging activity of plants was may be due to the presence of some important chemical compounds like polyphenol, alkaloids, glycosides, flavonoids, and steroids [15]. These phytochemical compounds were commonly found in plants have been reported to have multiple biological effects [16], including antioxidant activity. The Smilax china *extracts* contain different phytochemical constituents like alkaloids, phenols, flavanoids, glycosides etc. The methanolic Extracts showed better scavenging activity compared to other extracts and contain more phenolic and alkaloid contents. By the above results the scavenging activity of Smilax china *was* may be due to the presence of some important chemical compounds like phenols, alkaloids, glycosides, flavonoids, and steroids. The Smilax china extracts have scavenging activity against DPPH radicals. The isolation and characterization of active molecules (compounds) responsible for antioxidant activity work is in progress.

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