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In-Vitro Antioxidant Potentials and Free Radicals Scavenging Activity of *Solanum Incanum* L.

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Abstract
In recent trends in biomedical technology consist of growing clinical demands researcher to searching for new suitable therapeutic compound to direct towards to utilize of Biodiversity. However, the present scenario plant-based antioxidant can be effectively replacing the modern medicine. Although, *Solanum incanum* L., is roadside weed shrub and its ripened berries were treating throat like sore throat, angina, stomach-ache, painful menstruation, liver pain, pneumonia and rheumatism. In the present investigation carried out the antioxidant potentials of *Solanum incanum* for their free radical – scavenging activities was evaluated by Hexane, Chloroform, Ethyl acetate and methanol extracts by terms of DPPH free radical scavenging activity, Hydroxyl radical (OH•) – scavenging activity, Total Antioxidant activity. The estimation of crude extracts (Conc. 50, 150,250 and 500 µg/ml) by the IC_{50} value were calculated, compare to standard antioxidant for L-Ascorbic acid. All above mentioned the solvent extracts were analysed and result were concluded that the Hexane extract shows maximum free radical scavenging potentials compare the standards L-Ascorbic acid. However, total Phenol content (8.75±0.38mg/ml) Gallic acid equivalent (GAE), Total Flavanoid content (10.36±0.55 mg/ml) Quercetin (QE) equivalent were found higher in Hexane extracts. In the present investigation revealed that S.*incanum* can be a valuable source of natural antioxidants with their potential utilization in different fields of biomedical industrial application.

Keywords
Solanum incanum, free radical scavenging, antioxidant, Phenol, Flavanoid.

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INTRODUCTION
In the beginning of the 20th century scientist has been able to prove a common link among the various chronic diseases as well as actual aging process it may be caused by a phenomenon known as oxidative stress or free radical damage. Hence, based on this scenario the growing clinical demands on motivating the biomedical technology to searching for the new therapeutic compounds. However, the potentials of egg plants having a suitable alternative for various ailments, clinically useful therapeutic compound and their antioxidants potential for low-cost production of high quality much safer and biologically active. In recent years there is an upsurge in the plant antioxidants areas related to emerging developments in prevention of disease especially the

role of free radicals scavenging activity. (Devasagayam *et al.,* 2004) Moreover, antioxidants Studies till date have demonstrated and *in vivo* application of such potential Natural antioxidants activities in reducing oxidative stresses for potent and cost – effective manner. Natural antioxidants for various medicinal and aromatic plants having their own therapeutic potentials. Antioxidants are free radical scavengers, which can be prevent or protect, stop or reduce the generation of free radicals. The impacts of free radicals can be reduced by endogenous defence systems. In general, have the immune system is vulnerable to oxidative stress, as the innate defence is not enough against serve to continuous oxidative stress. During such free radicals affected conditions, there is need to boost the antioxidant abilities. Hence, the source of compounds which are natural resources need to be identified for having potency to prevent ROS (induced) damage. (Farrukh Aqil *et al* 2006).

The *Solanum incanum* is rich natural antioxidants in the form of alkaloids, phenolics, flavonoids, sterols, saponins, glycosides and carbohydrates, Vitamins, fatty acids, tannins and amino acids. Screening of the whole plant is done by how much amount of measuring the antioxidant activity through various *invitro* models like, DPPH methods, Hydroxyl radical ($OH^{\bullet}$) – scavenging activity, Total antioxidant activity, Total Phenol content and Total Flavonoid Contents. Solanaceae is the biggest and most complex comprising 85 genera and 2800 species. *Solanum incanum* L. Synonyms: *Solanum panduriforme* E. Mey. *Solanum bojeri* Dunal. Vernacular Name is Mullakathirikkai, with Common name is Indian nightshade, thorn apple, bitter apple, bitter ball and bitter tomato. Itis a nightshade family that is native to sub – Saharan Africa and grown in many regions of Far East Asia, Africa, and Middle eastwards to India. The Plant is an erect or spreading perennial shrub small prickle in leaves and stems. They creepy in nature, leaves are arranged in alternate, usually simple and lack stipules. In nature, the flowers are actinomorphic of only slightly zycomorphic. The young green and ripened fruits are yellow coloured. It is found that effectively systemically in treating throat like sore throat, angina, stomach-ache, painful menstruation, liver pain, pneumonia and rheumatism and it is having antimicrobial and anti-tumour activities. In the present study, we have made attempt to antioxidant potentials of *Solanum incanum* in for their free radical – scavenging activities, via DPPH, Hydroxy radicals Scavenging activities, Total Phenol content and Total Flavanoid content.

MATERIALS AND METHODS

Source of Plant

An intensive survey to be conducted on during flowering season for July to October 2014. The plant *Solanum incanum* L. were collected from Southern Western Ghats, exactly, Sangarangudi forested tribal hamlet (Very Near to Nallamudi Veiw point; Anamudi settlement is an adjacent hamlet) Valparai taluk, in the Coimbatore district of Tamil Nadu, India. This place located at 10^{o} 24'75.25'' N 76^{o} 93'73.70'' E., and above sea level is 3,500 feet (1,462 m) on the Annamalai Hills range ecoregions of the Western Ghats, at the distance of 100 kilometres (62 mi) from Coimbatore and 65 kilometres (40 mi) from Pollachi (with include 40 hairpin bends via Aliyar). The plant collected from dense forest continues to be out of bounds to Nallamudi estate. *Solanum incanum* is rare medicinal plant, the plant collected from Wild, forest Roadside land habitat. Where the natives gather and consume a good condition of plants and standard method was followed with regard to collection of plant materials, drying, mounting, preparation and preservation of plant specimens and maintain voucher specimens in minimum triplicates were collected due to similar method was made. (Jain and Rao, 1976).

Identification and Authentication of Plant materials:

The plant species were initially identified and got specimen accession number is AUBOT#242 with reference to Gamble,1935; Fischer, C.E.C., 1921 and herbarium voucher specimen was deposited at Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu. Further, identified and authenticated By Dr. G.V.S Murthy, Scientist 'F' & Head of Office, Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore. Herbarium Voucher Number is BSI/SRC/5/23/2015/Tech. Dated July 7^{th}, 2015.

Reagents

All the Chemicals were purchased from spectrochem PVT. Ltd., India. such as 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), Hexane, Chloroform, Ethyl acetate, Methanol, Ascorbic acid, Deoxyribose, $FeSO_4.7H_2O$. Sodium Phosphate Buffer, $H_2SO_4.7H_2O$, Trichloroacetic acid, Thiobarbituric acid, Sulphuric acid, Sodium Phosphate, Ammonium molybdate, Folin – Ciocalteu reagent, Sodium carbonate, $NaNO_2$, $AlCl_3$, NaOH, Gallic acid, and Quiertine were purchased from E. Merck (india) Limited. All the reagents were analytical grade.

Preparation of crude plant extracts

The source of collected plant were brought into the laboratory, The plants samples were precleaned, washed with running water, then surface sterilized with 10% sodium hypochlorite solution rinsed with sterile distilled water, shade dried under room temperature then dried hot – beaming over at 50°C, water contents reduce below 10% they were cut into small pieces finally ground into a coarse powder in a mechanical blender and kept at room temperature prior to extraction. The extract was prepared using soxhlet extraction apparatus by different solvent systems were used, owing Hexane, Chloroform, Ethyl acetate and Methanol. (Anonymous, 1966; Beecher et al., 2004.) The crude extract samples were stored in below 20°C to maintain a under the desigator. These samples were making different concentrations ranges from 50, 100, 150, 250, and 500mg/ml and it is utilized for the various analyses of Antioxidant potentials activities of *Solanum incanum.*

(i) DPPH free radical – scavenging activity

In 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity evaluated by the method followed by Blios (1958); the crude extracts sample (0.1ml) at various concentration (50, 150, 250 and 500µg/ml) were using for this antioxidant was added to a 0.004% MeOH solution of DPPH. The reaction mixture was incubated for 20min at 28ºC in the dark place. The optimized control sample contains which all the reagent mixture without plant sample and it was as blank, free radical scavenging effect of *Solanum incanum* for extracted in different organic solvents, ascorbic acid as a standard and inhibition activity were estimation. Further absorbance at 517 nm was determined after 30 minutes performed by spectrophotometer (Hitachi U-2001). Similarly, all the tests were performed in triplicate. The antioxidant activities of plant extract were expressed as IC_{50} value, which was defined as the concentration (µg/ml) of extracts required to inhibit the formation of DPPH radicals by 50 percent. The DPPH radical concentration was calculated using the following formula

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

(ii) Hydroxyl radical (OH•) – scavenging activity

The hydroxyl radical scavenging activity was carried out using the 2' - deoxyribose oxidative degeneration assay according to Elizabeth and Rao, 1990. The different concentrations (50, 150, 250 and 500) of plant extract were prepared, and the reaction mixture contained 0.1ml of deoxyiribose, 0.1ml of $FeCl_3$, 0.1 ml of EDTA, 0.1 ml H_2O_2, 0.1 ml of ascorbate, 0.1ml KH_2PO_4 –KOH buffer of plant extracts in a final volume of 1.0 ml. The mixture was incubated at 37ºC for 1 hr. At the end of the incubation period, 1 ml of TBA was added and heated at 95ºC. After cooling, the TBA formation was measured spectrophotometer (Hitachi U-2001) at 532nm against an appropriate blank. The hydroxyl radical scavenging activities were determined by comparing the absorbance of the control with prepared samples. The TBA production for positive ascorbic acid fixed 100% and the relative percentage of TBA was calculated by the extract.

$$\text{Hydroxyl radical Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

(iii) Total antioxidant activity

The total antioxidant capacities for the analysed samples of *S. incanum* were formed by **Prieto *et al.*, (1999).** In brief of analysis to 2 mg/ml of sample were taken and mixed with 1 ml of reagent solution (0.6 M Sulfuric acid, 28mM Sodium phosphate and 4 mM ammonium molybdate followed by incubation at 95º C for 90 Minutes. After samples were cooled to 25º C the absorbance was measured for 695nm against a blank. The blank contained 1ml of the reagent solution were making without the sample. The total antioxidant activity was expressed in form of ascorbic acid equivalents, and it was carried out in triplicate.

$$\text{Total antioxidant activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

(iv) Total phenol content (TPC)

The total phenolic content of S. *incanum* in different solvents extract and determined by the way of Singleton et al., (1999). In this method, reaction mixture contained 0.5 ml of Folin – Ciocalteu reagent and allowed to stand at 22°C for 5 minutes; 7.5% Na_2CO_3 and 0.5 ml of the plant extract mixed thoroughly. After 2 h of incubation at room temperature, the

absorbance of the reaction mixture was measured in 765 nm after the mixer was stirred and allowed to stand for 30 minutes. The results were expressed in the form of Gallic acid equivalents (GAE). All the tests were performed in triplicate.

(v) Total flavonoids content (TFC)

The flavonoids content was determined by aluminium trichloride method using Catechin as a reference compound. (Chang *et al.,* 2002). This method based on the formation of a complex flavonoid – aluminium having the absorptive spectrophotometer (Hitachi-U-2001) maximum at 415nm, after remained react at room temperature for 30min. Briefly, 0.5 ml of each solvent extracts (1:10g/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1ml of 1m potassium acetate and 2.8 ml of distilled water. All the value was express as following Quercetin equivalent (QE)/100 g of extract. Each assay was triplicate.

Statistical analysis

All the experiments were performed in triplicate (n=3) and results were expressed as mean±SD. Statistical analysis was carried out with (SPSS Inc., Chicago, IL, USA. package version 16.0 Statistical Software) Using ANOVA followed By Duncan's Test ($P<0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

(i) DPPH free radical – scavenging activity

The *Solanum incanum* crude extracts were tested, by different solvents extracts. Among the solvents, methanol extract expressed the higher DPPH radical scavenging activities shown Fig.No.1. and its IC_{50} Value is (64.721µg/mL) higher than Ethyl acetate (104.21µg/mL), Chloroform (154.028µg/mL), Hexane (411.485 µg/mL) and lower value of activity were observed Ascorbic acid (20.221µg/mL). At 100 mg/ml, the result shows that the scavenging activity of different solvents IC_{50} values on DPPH of *S. incanum* higher activity compared to ascorbic acid in the order of methanol> ethyl acetate > chloroform > hexane > ascorbic acid. (Table.No.1) the result obtained in this investigation revealed that the DPPH radical scavenging activities of *Solanum incanum* might be attributed to the hydrogen donating ability.

Table. 1. DPPH Radical Scavenging Activity of *S. incanum* crude extracts

Solvents	Concentrations (mg/ml)				IC50 Value(µg/ml)
	50	150	250	500	
Hexane	29.54±0.13	39.20±0.14	52.18± 0.98	71.20±0.50	411.485
Chloroform	38.64±0.19	48.70±0.50	61.31±0.28	78.68±0.76	154.028
Ethyl acetate	45.35±0.50	52.60±0.33	65.38±0.78	84.12±0.28	104.21
Methanol	49.20±0.20	58.78±0.78	73.88±0.76	94.30±0.50	64.721
Ascorbic acid	54.17±0.12	67.16±0.32	78.70±0.05	98.28±0.78	20.221

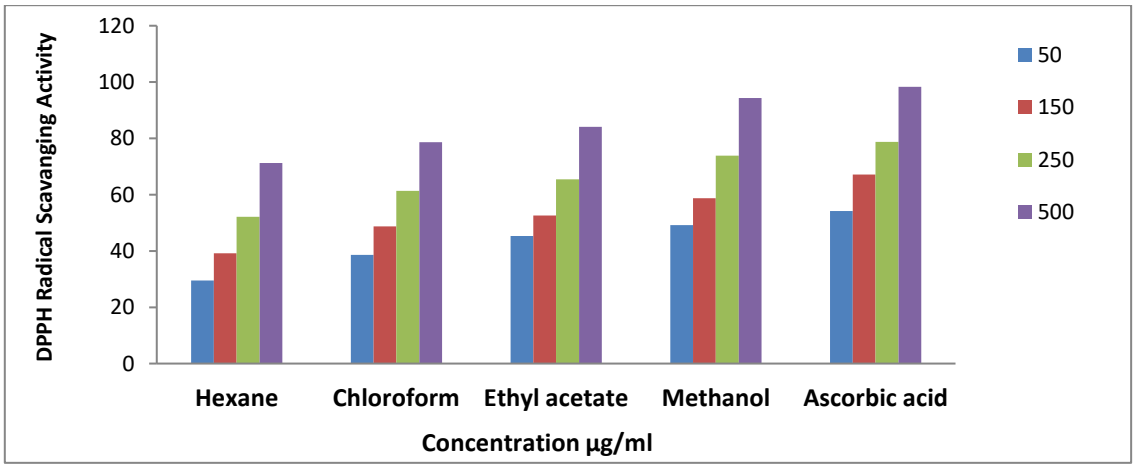

Fig.1. DPPH Radical Scavenging Activity of *S. incanum* crude extracts

The effect on DPPH radical scavenging activity effect found that aromatic amines. (e.g., p-phenylenediamine, p-aminophenol) and polyhydroxy aromatic compounds (hydroquinone, pyrogallol, gallic acid) due to hydrogen donating antioxidant, which react with DPPH and reduction of methanolics solution of stable diamagnetic molecule by discoloration of the formation of the non-radical

form of α, α-diphenyl-β-picryl Hydrazine. (Deepa Babu *et al.*, 2013); Moreover, the scavenging effect increased with increasing concentration of the extract. The DPPH is a commercially available oxidizing radical scavenger. It can be used to study the reaction kinetics of various antioxidants, quantify and to compare the free radical scavenging capacity of different antioxidant (YU-2001). From the methodological point of view the DPPH methods is recommended as easy and accurate with regard to measuring the antioxidant activity of fruit and vegetable extracts. (Gil *et al.,* 2000).

(ii) Hydroxyl radical (OH•) – scavenging activity

The *Solanum incanum* crude extracts is tested, by different solvents extracts. Among these solvents, Methanol extract exhibited the highest Hydroxyl radical scavenging activities calculated Fig.No.2. The IC_{50} Values of Methanol is consists of higher (313.676 µg/ml) than Ethyl acetate (413.304µg/ml), Chloroform (721.648µg/ml), Hexane (773.678µg/ml) and Ascorbic acid (80.422µg/ml). At 100 mg/ml, the result shows (Fig No.2) that the Hydroxyl scavenging activity of IC_{50}values on different solvents extracts of *S. Incanum* decreases than that of standard in the order of Methanol > Ethyl acetate > Chloroform >Hexane > Ascorbic acid. (Table.No.2)

Table.2. Hydroxyl radical scavenging activity of *S. incanum* Crude extracts

Solvents	Concentrations (µg/ml)				IC50 Value (mg/ml)
	50	150	250	500	
Hexane	13.63±0.06	18.42±0.03	21.32±0.07	29.59±0.22	773.6
Chloroform	17.21±0.51	19.43±0.06	25.42±0.1	35.31±0.12	721.648
Ethyl acetate	19.48±0.23	27.69±0.08	34.78±0.06	57.35±0.03	413.304
Methanol	23.05±0.32	34.14±0.15	48.27±0.02	65.94±0.59	313.676
Ascorbic acid	45.13±0.35	61.07±0.01	76.36±0.13	91.09±0.72	80.422

The scavenging ability of *Solanum incanum* soluble fractions have consist of the degradation of deoxyribose by OH• released certain products, which upon heating condition. *Solanum incanum* crude extracts exerted inhibition of OH formation during incubation period and percentage of inhibition is higher than ascorbic acid equivalents. Hydroxyl radical is highly reactive oxygen, formed from the reactions of various hydroperoxides with transition metal ions. It attacks protein, DNA, polyunsaturated fatty acid membranes and most biological molecules it contacts and is known to be capable of abstracting hydrogen atoms from membrane lipids and brings about peroxide reactions of lipids. (Lie. *et.al.,* 2005).

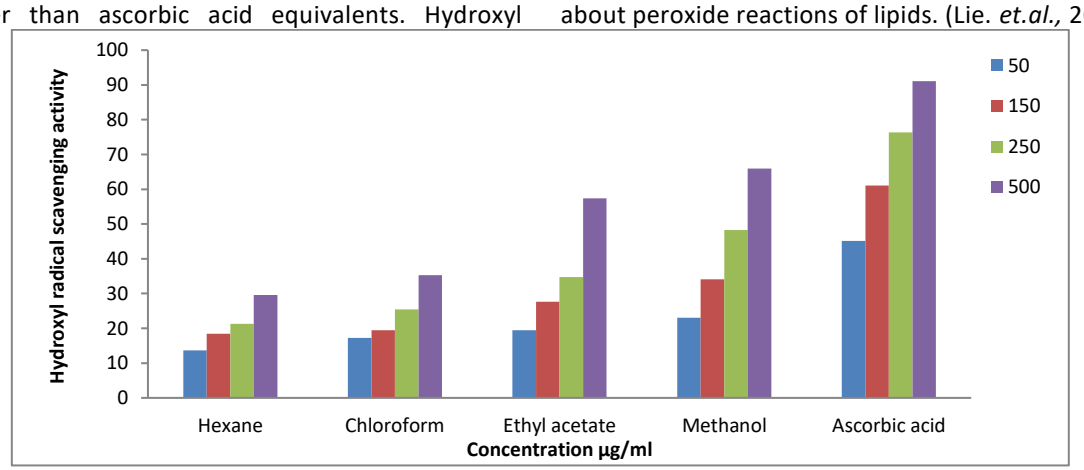

Fig. 2. Hydroxyl radical scavenging activity of *S. incanum* crude extracts

(iii) Total antioxidant activity

(Phosphomolybdenum method)

All the fractions of *Solanum incanum* crude extracts showed a significant total antioxidant activity shown Fig.No.3. Total antioxidant capacity was also determined in these extracts i.e., Hexane, Chloroform, Ethyl acetate, Methanol and ascorbic acid. Among the *Solanum incanum* different solvents extracts tested and their IC_{50} value viz., Methanol (99.737µg/ml) exhibited the higher Total antioxidant capacity, followed by Ethyl acetate (147.108µg/ml), Chloroform (259.131µg/ml), Hexane extract

(274.924µg/ml) and lower IC_{50} value of absorption was noted in Ascorbic acid. (64.032µg/ml). At 100 mg/ml, the result shows that the scavenging activity of values on Total antioxidant capacity of different solvents of S. *incanum* extracts decreases in the order of Methanol > Ethyl acetate > Chloroform > Hexane > Ascorbic acid. (Table.3)

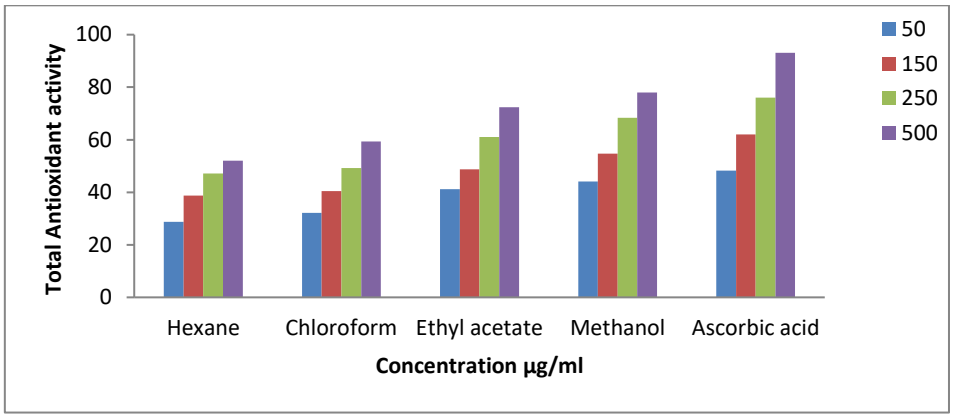

Fig. 3. Total Antioxidant activity *S. incanum* Crude extracts

Table.3. Total Antioxidant activity *S. incanum* Crude extracts

Solvents	Concentrations (µg/ml)				IC50 Value (mg/ml)
	50	150	250	500	
Hexane	28.72±0.17	38.75±0.50	47.18±0.78	52.00±0.17	274.924
Chloroform	32.23±0.12	40.48±0.04	49.20±0.50	59.32±0.27	259.131
Ethyl acetate	41.12±25	48.72±0.16	61.00±0.05	72.32±0.27	147.108
Methanol	44.05±0.73	54.72±0.36	68.38±0.78	78.01±0.28	99.737
Ascorbic acid	48.20±0.05	62.07±0.61	76.05±0.13	93.09±0.72	64.032

On the other hand, determination of specific antioxidant species might be less useful than the knowledge of the total antioxidant capacity of a prepared sample. In the case when one wants to analyze the antioxidant contributions of specific dietary components and how this relates to the antioxidant composition and activities of the individual constituents. Other situations where the knowledge of total antioxidant activity can be useful to include the analysis of changes in plasma antioxidant activity related to oxidative stress, or the understanding of structure–activity relationships of pure antioxidant species. Because of its simplicity and the cheap reagents, it uses, the phosphomlybdenum method as equivalents of ascorbic acid. E.g., Four extracts of *Enicostemma axillare* was examined for *in vitro* antioxidants activity with half maximal inhibitory concentrations (IC_{50}) values, ranged from 13.26 to 24.36µg/ml. All extracts showed the moderate antioxidants capacity using the phosphomlybdenum method the observed results indicated by (Jaishree *et al.,* 2008).

(iv) Total phenolic content (TPC)

The higher total Phenol content of *Solanum incanum* was find Methanol (1.94±0.76µg/ml), Ethyl acetate (4.76±0.78µg/ml), Chloroform (6.38±0.73µg/ml), Hexane extracts (8.75±0.38µg/ml), followed by and Gallic acid (1.75 ± 0.38µg/ml). Fig.No.4. At 100 mg/ml, the result shows that ofIC_{50} values on Total Phenolic Content of different solvents of S. *incanum* extracts decreases in the order of Methanol > Ethyl acetate > Chloroform Hexane > Gallic acid. (Table.No.4)

Table. No. 4. Total Phenol and Total Flavonoid of *S. incanum* crude extracts

Solvent	*Solanum incanum* crude extracts	
	Total Phenol content (50µg/ml) Gallicacid(GAE)	Total Flavanoid content (50µg/ml) Quercetin (QE)
Hexane	8.75±0.38	10.36±0.55
Chloroform	6.38±0.73	6.34±0.18
Ethyl acetate	4.76±0.78	4.35±0.65
Methanol	1.94±0.76	3.25±0.39
Standards	1.75±0.50	2.98±0.52

Phenols are very important major role of plant constituents because of their radical Scavenging ability due to their hydroxyl groups. The phenolic content may contribute directly to the antioxidative action. Most of the work dealing with phenolic content in natural products use Gallic acid as standards (independently of phenolic species detected), the content of phenolic in this work was expressed as gallic acid equivalents (GAE) to facilitate comparison (Jerez *et al.,* 2004). The phenolic compounds are considered the major determinants for the antioxidant capacity in plants (Velioglu *et al*., 1998: Dorman *et al.,* 2003). Plants are the source of the most potent free radical scavengers such as phenolic compounds and Vitamins. Therefore, the investigations of biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are numerous in the recent studies (Albayrak and Aksoy, 2010 : Sales *et al.,* 2010).Italian researchers reported the strong antioxidant properties of *Rubus ulmifolius* leaves attributing them to the activity of phenolic compounds like caffeic acid, ferulic acid, and caffeicquinic esters as well as quercetin 3-O-glucuronide,kaempferol-3-O-glucuronide (Dall'Acqua*et al*., 2008) and to ellagic acid (Martini *et al*., 2000).

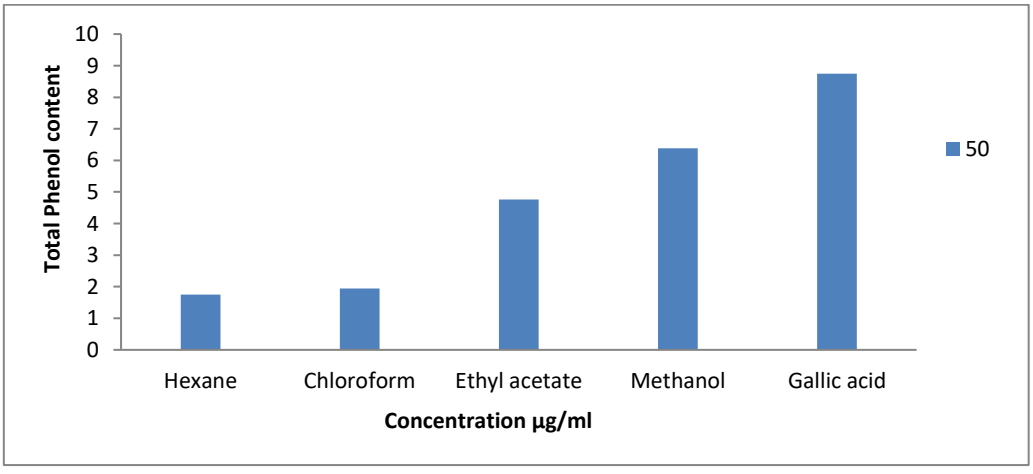

Fig. 4. Total Phenol of *S. incanum* crude extracts

(v) Total flavonoids content (TFC)

The flavonoids content (µg/ml) in evaluated by Quercetin as equivalents. The highest amounts of flavonoids were found in extracts of *S. incanum* contained Methanol (3.25± 0.39 µg/ml), Ethyl acetate (4.35±0.65 µg/ml), Chloroform (6.34±0.18 µg/ml), Hexane (10.36±0.55µg/ml), followed by Quercetin (2.98±0.52 µg/ml) respectively. (Fig.No.5) It is known that only Flavonoids of a certain structure and especially hydroxyl position in the molecule determine antioxidant properties. In general, these properties depend on the ability to donate hydrogen or electron to a free radical, the result shows that the scavenging activity of values on total Flavanoid content of different solvents of *S. incanum* decreases in the order of Methanol > Ethyl acetate > Chloroform > Hexane > Quercetin. (Table No.5).

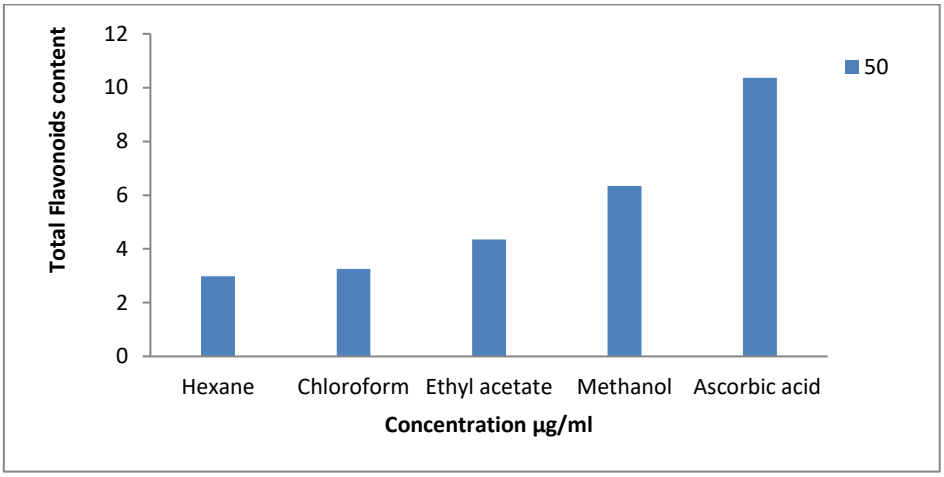

Fig. 5. Total Flavonoid of *S. incanum* crude extracts

Flavonoids as one of the most diverse and widespread group of natural compounds. (Adaikalaraj *et al*., 2017). These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties (Miliauskas *et al*., 2004). Antioxidant activity depends upon the number and positions of hydroxyl groups, other substituent and glycosylation of Flavonoid molecules. Depending on their structure, flavonoids are able to scavenge practically all known ROS (Bouazi*et al*., 2005). The observation also helping the suitable potentially analysed antioxidant activity of medicinal plants is due to phenolic or flavonoid content (Owen *et al*., 2003; Mariani *et al*., 2008). Quercetin (Ordonez *et al.,* 2006) can be used as a positive control in total Flavonoid content estimation experiments.

CONCLUSION

In summary the plant antioxidants having incredible potentials. Among the *S. incanum*, the crude extracts and their compounds are gaining interest as antioxidant and widely accepted because of their relatively high volatility, epidermal nature and biodegradability. On the basis of the results obtained in the present study, it is concluded that free-radical-scavenging system revealed that the *Solanum incanum* had significant antioxidant activity were noteworthy in methanol extracts. The free radical – scavenging property have one of the mechanisms by this drug delivery system useful as foodstuff as well as a traditional medicine. However, further investigation of individual compound, there *in vivo* antioxidants activities to facilitate it for clinical trials and in different antioxidant mechanisms is warranted. In that way, *Solanum incanum* can be a valuable source of natural antioxidants with their potential utilization in different fields and its suitable potential application of biomedical industry.

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