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An analysis of Anti-inflammatory activity of methanolic extract of leaves of *Solanum virginianum* using-carrageenan induce Paul edema model

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Abstract

Solanum virginianum (Family: Solanaceae) commonly known as the Indian night shade or Yellow berried night shade (English) and kantakari (Sanskrit). It is a prickly diffuse, bright green perennial herb, woody at the base, 2–3 m height, found throughout India, mostly in dry places. *Solanum virginianum* has as expectorant and antipyretic activities.

The methanolic extract of leaves of *Solanum virginianum* is administered orally to a group of experimental animals at one of the defined doses such as 5, 50, 500, 2000mg/kg. There was no sign of toxic effect in dose between 5, 50, 300mg/kg. When the dose of 2000 mg/kg given to the animal, the toxic effect on animal was found such as low breathing, itching, swallowing and mortality. Then, 1/3 of the dose has been selected which is 100 mg/kg.

The anti-inflammatory activity was evaluated using carrageenan induced paw edema models in wistar albino rats. The anti- inflammatory activity was found to be dose dependent in carrageenan-induced paw edema model. The results of present study demonstrate that methanolic extract of the leaves of *Solanum virginianum* possess significant ($P < 0.05$).

The amount of the plants which have been asserted to possess anti-inflammatory effect is so much that evaluating all of them are out of the scope. Herbal medicine is one of the most important aspects of complementary medicines. There are many studies which have been asserted the role of several herbs in inflammation remission.

Keywords: *Solanum virginianum,* phytochemical screening, antioxidant activity, DPPH, TPC, TFC, anti-inflammatory activity, carragenan induced paw edema

1. Introduction

Ayurvedic medicines mainly based on plants enjoy a respective position today, especially in the developing countries, where modern health services are limited. Safe effective and inexpensive indigenous remedies are gaining popularity among the people of both urban and rural areas especially in India and China. Information from ethnic groups or indigenous traditional medicines has played vital role in the discovery of novel products from plants as chemotherapeutic agents [1].

In India, around 15000 medicinal plants have been recorded [6] however traditional communities are using only 7,000 - 7,500 plants for curing different diseases [7-9]. The medicinal plants are listed in various indigenous systems such as Siddha (600), Ayurveda (700), Amchi (600), Unani (700) and Allopathy (30) plant species for different ailments [10]. According to another estimate 17,000 species of medicinal plants have been recorded out of which, nearly 3,000 species are used in medicinal field [11]. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants [12-13]. The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, alkyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters [2].

Among them some are act as synergistic and enhance the bioactivity of other compounds. Artemisinin produced by *Artemisia annua* plant is very effective against *Plasmodium falciparum*, *P. vivax* and also drug resistant parasite. The main active constituents of *Artemisia annua* are sesquiterpenoid lactone endoperoxides named artemisinin and artemisinic acid. For more than century quinine, an alkaloid obtained from the bark of various species of cinchona trees has been used in the treatment of Malaria and interestingly was one of the first agents used for the treatment of amoebic dysentery.

Reserpine isolated from raw plant extract of *Rauvolfia serpentina* is used as tranquilizer and in control of high blood pressure. From 2000 years the powdered root of Rauvolfia *serpentina*

has been used in treatment of mental illness in India. Although synthetic drugs are often used in treatment of certain disease but a remarkable interest and confidence on plant medicine was found [15]. Indian Vedas describe the widespread use of herbal products and aqueous extract of different plant parts for curing different diseases. Maximum 30 % of root part of medicinal plant is used in different practices in comparison to other plant parts [3].

1.1 Antioxidant

An antioxidant is a chemical that prevent the oxidation of additional chemicals. They save from harm the key cell components by neutralizing the injurious effects of free radicals, which are natural by- products of cell metabolism [85-86]. Free radicals form when oxygen is metabolized or twisted in the body and are substance class that posses an unpaired electron in the outer (valance) shell of the molecule. This is the rationale, why the free radicals are greatly reactive and can react with proteins, lipids, carbohydrates and DNA. These free radicals attack the nearby stable molecules, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, opening a chain reaction, lastly resulting in the description of a living cell [4].

Free radicals might be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS, reactive nitrogen species). The oxygen derived molecules are O^-_2[superoxide], HO [hydroxyl], HO_2 [hydroperoxyl], ROO [peroxyl], RO [alkoxyl] as free radical and H_2O_2oxygen as non-radical. Nitrogen derived oxidant species are mainly NO [nitric oxide] ONOO [peroxy nitrate], NO_2 [nitrogen dioxide] andN_2O_3 [dinitrogen trioxide] [5-6].

In a typical cell, there is suitable oxidant: antioxidant balance. However, this balance can be shifted, when manufacture species is increased or when levels of antioxidants are diminish. This phase is called oxidative stress. Oxidative stress results in the injure of biopolymers as well as nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates. Lipid peroxidation is oxidative deterioration of polyunsaturated lipids and it involves ROS and transition metal ions. It is a molecular mechanism of cell injury leading yield a wide range of cytotoxic products, most of which are aldehydes, like malondialdehyde (MDA), 4- hydroxynonenal (HNE), Oxidative stress causes serious cell damage leading to a variety of human diseases [7] like Alzheimer’s disease, Parkinson’s disease, atherosclerosis, cancer, arthritis, immunological incompetence and neurodegenerative disorders, etc. Nutritional antioxidant deficiency also leads to oxidative stress, which signifies the identification of natural anti-oxidative agents present in die obsessive by human population [8].

Table 1.1: List of *In-Vitro* antioxidant methods

S No.	Name of the method
I	Hydrogen Atom Transfer methods (HAT)
1.	Oxygen radical absorbance capacity (ORAC) method
2.	Lipid peroxidation inhibition capacity (LPIC) assay
3.	Total radical trapping antioxidant parameter (TRAP)
4.	Inhibited oxygen uptake (IOC)
5.	Crocin bleaching Nitric oxide radical inhibition activity
6.	Hydroxyl radical scavenging activity by p-NDA (p-butrisidunethyl aniline)
7.	Scavenging of H_2O_2radicals
8.	ABTS radical scavenging method
9.	Scavenging of super oxide radical formation by alkaline (SASA)
II	Electron Transfer methods (ET)
1.	Trolox equivalent antioxidant capacity (TEAC) decolourization
2.	Ferric reducing antioxidant power (FRAP)
3.	DPPH free radical scavenging assay
4.	Copper (II) reduction capacity
5.	Total phenols by Folin-Ciocalteu
6.	N,N-dimethyl-p-Phenylenediamine (DMPD) assay

2. Plant Profile

Fig 2.1: *Solanum Virginianum L*

2.1 Botanical classification

Table 2.1: Botanical Classification

Kingdom	Plantae
Division	Spermatophyta
Sub- Division	Angiospermae
Class	Dicotyledonae
Sub-class	Gamopetalae
Series	Bicarpellatae
Order	Polemoniales
Family	Solanaceae
Genus	*Solanum*
Species	*Virginianum. L*

2.2 Phytochemistry/Chemical Constituents

Steroidal alkaloid solasodine is the principal alkaloid. Alcoholic extracts of the plant contain fatty and resinous substances. Solasonine is present in fruits. Fruits contains solasonine, solamargine, solanocarpine, beta –solamargine and solanocarpidine. Dry fruits contain traces of

isochlorogenic, neochronogenic, chronogenic and caffeic acids. Petals yielded apigenin, stamens gave quercetin diglycoside and sitosterol. The unsaponifiable matter of fruit contain two sterols, one of which is carpesterol [9]. Tupkari et.al [71]. Noted the presence of coumarins, scopolin, scopoletin, esculin and esculetin from plant parts of *S. xantocarpum*; constituents were separated through column chromatography Kusano *et.al* [10] reported the following steroidal constituents namely, cycloartanol, sitosterol, stigmasterol, campesterol, cholesterol, glucoside, solamargine and beta-solamargine from fruit extracts.

Table 2.2: Medicinal uses of different parts of *Solanum Virginianum* L.

S No.	Parts	Treatment
1.	Fruits	anthelmintic, antipyretic, laxative, anti-inflammatory, anti-asthmatic and aphrodisiac activities, treating pimples and swellings
2.	Fruit juice	used in sore throats and rheumatism; decoction of the plant is used in gonorrhea
3.	Leaves	cough and asthma
4.	Roots	Expectorant and diuretic, fever, coughs, asthma and chest pain.

3. Experimental work

3.1 Selection of plants for the study

Ethnobotanical surveys were conducted in different tribal localities of Madhya Pradesh. The method adopted for collection of data was interview with tribals, local medicine men and one to one discussion about therapeutic use of local plants in the treatment of various diseases. Present work carried out on plant species *Solanum Virginianum.*

3.2 Collection of plant material

Leaves of plant of *Solanum virginianum* was collected from rural area of Bhopal (M.P), India in the months of January, 2018.

3.3 Preparation of plant material for study

Plant materials (leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time. Then these plants materials were shade dried without any contamination for about 3 to 4 weeks. Dried plant materials were grinded using electronic grinder. Powdered plant materials were observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container till any further use.

3.4 Extraction by maceration process

Dried powdered *Solanum Virginianum* has been extracted with methanol solvent using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

Flow chart of extraction method

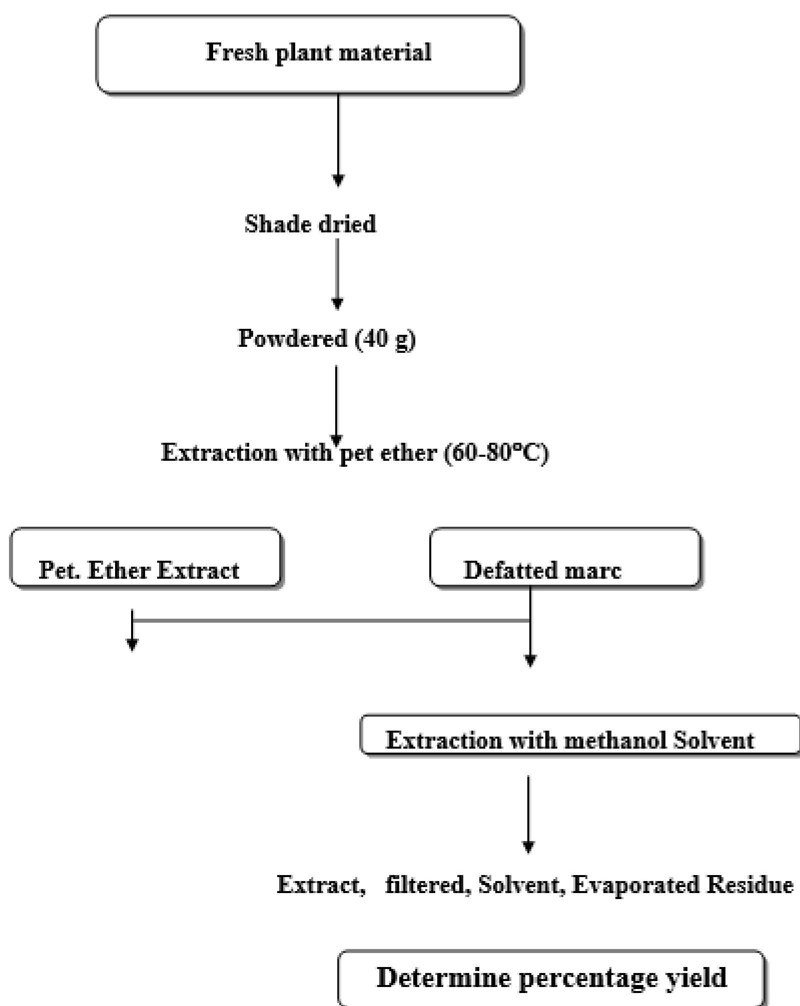

Fig 3.2: Flow chart of extraction procedure

3.5 Total Phenolic content estimation [11]

Principle: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol

Preparation of Extract: 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of Phenol.

Procedure: 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

3.6 Total flavonoids content estimation [12]

Fig 3.3: Standard Preparation of Flavonoids

Principle: Determination of total flavonoids content was based on aluminium chloride method

Preparation of Standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of Extract: 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

3.7 *In-vitro* free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl - DPPH) [13]

It is a **s**table free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.

Fig 3.4: Antioxidant test

Procedure

DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6mg in 100 Methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance of 0.886. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes.

Protocol for DPPH Free radical scavenging activity

a) Preparation of stock solution of standard or test sample: 10 mg of the standard or extract was dissolved in 10 ml of methanol to get 1000 µg/ml solution.
b) Dilution of standard or test solution: 10, 20, 40, 60, 80 and 100µg/ml solution of the standard or test samples were prepared from stock solution.
c) Preparation of DPPH solution: 6 mg of DPPH was dissolved in 100 ml of methanol. The final solution was covered with aluminum foil to protect from light.

Estimation of DPPH radical scavenging activity

- 1.5 ml of DPPH solution was taken and 1.5 ml with methanol, absorbance was taken immediately at 517 nm for control reading.
- 1.5 ml of DPPH and 1.5 ml of the test sample or standard of different concentration were put in a series of volumetric flasks. Three test samples were taken and each processed similarly. Finally the mean was taken.
- Absorbance at zero time was taken for each concentration.
- Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

$$\text{Calculation of \% Reduction} = \frac{\text{Control Absorbance - Test absorbance} \times 100}{\text{Control Absorbance}}$$

4. Results and Discussion

4.1 Determination of extractive value of leaves of *Solanum Virginianum*

Table 4.1: % Yield of *Solanum virginianum*

S. No.	Parts	Methanol % extractive value(W/W)
1.	Leaves	2.6

The extractive value of extracts of *Solanum virginianum* was investigated and represented in Table No. 7.1. From the present study it was found that, the extractive value of *Solanum virginianum* in methanolic extract was 2.6%.

4.3 Results of Estimation of Total Phenolic & Total flavonoid Contents

4.4 Total Phenolic content estimation (TPC)

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve**:** Y = 0.042X+0.002, R^2= 0.999**,** where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Calibration curve of Gallic acid

Table 4.2: Preparation of calibration curve of Gallic acid

S. No	Concentration	Absorbance
0	0	0
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035

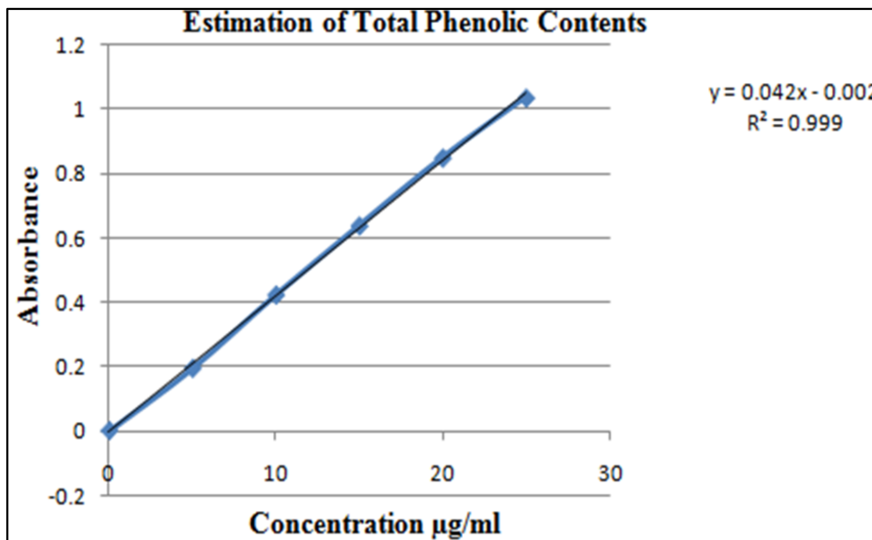

Fig 4.1: Graph of Estimation of Total Phenolic content

4.5 Total flavonoid content estimation (TFC)

The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.06X+0.019, R^2= 0.999, where X is the quercetin equivalent (QE) and Y is the absorbance.

Calibration curve of quarcetin

Table 4.3: Preparation of calibration curve of Quarcetin

S. No.	Concentration	Absorbance
0	0	0
1	5	0.352
2	10	0.61
3	15	0.917
4	20	1.215
5	25	1.521

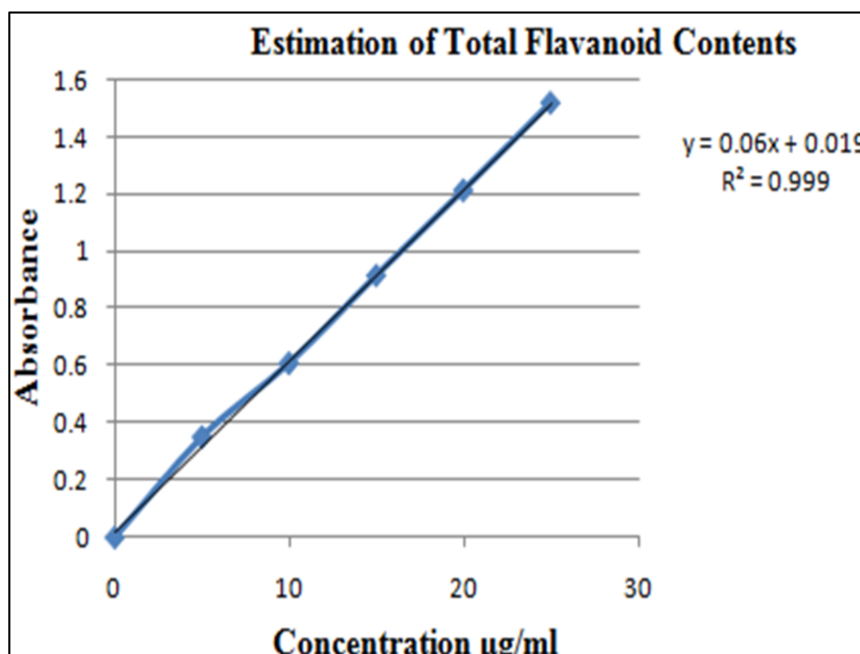

Fig 4.2: Graph of Estimation of Total flavonoid content

Table 4.4: Total Phenolic and Total flavonoid content of *Solanum virginianum*

S No.	Extracts	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE)(mg/100mg)
1.	Leaves (Methanol)	1.519	0.63

Total phenolics and Total flavonoid content of leaf extract also highest (1.519 mg gallic acid/100mg and 0.63 mg quercetin /100mg) which attributed to the antioxidant activity of *Solanum virginianum* leaf extract in this study.

4.6: DPPH radical scavenging activity

Table 4.5: DPPH Radical Scavenging Activity

S. NO.	Concentration (µg/ml)	Vitamin C(Ascorbic acid)		Methanolic Extract	
		Absorbance	% Inhibition	Absorbance	% Inhibition
1	10	0.669	24.49±0.22	0.674	23.92±0.76
2	20	0.469	47.06±0.37	0.622	29.79±0.83
3	40	0.234	73.58±0.28	0.452	38.82±0.18
4	60	0.106	88.03±0.19	0.483	45.48±0.32
5	80	0.051	94.24±0.14	0.289	67.38±0.48
6	100	0.046	94.8± 0.26	0.244	72.46±0.49
	IC_{50} Value		21.7±0.36		58.3±0.33

Absorbance of 0.01mM DPPH Solution =0.886

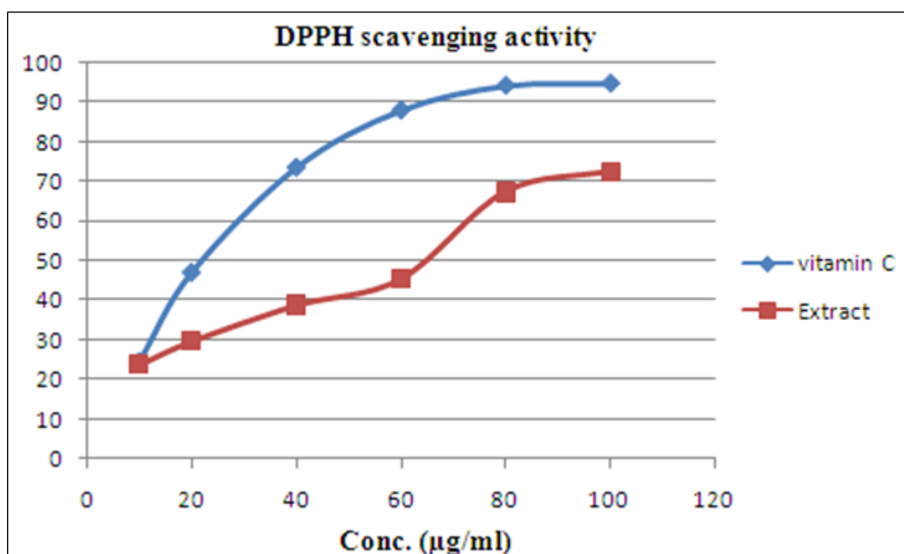

Fig 4.3: Graph of *In vitro* free radical scavenging activity

Quantitative DPPH radical scavenging assay Radical scavenging DPPH assay were conducted using the standard protocols. DPPH solution in methanol was prepared and 1.5 ml of this solution was added to 1.5 of extract solution (methanol) in methanol at different concentrations (10-100µg / ml). After 30 minutes incubation, the absorbance was measured at 517nm. Ascorbic acid at various concentrations (10-100µg/ ml) was used as reference compound. Lower the absorbance of reaction mixture indicates higher free radical scavenging activity. The capability to scavenge DPPH free radical was calculated using the following equation.

5. Conclusion

The phytochemical screening revealed the presence of carbohydrates, proteins, amino acids, flavonoids, saponins, diterpenes and phenolic compounds in the methanol extract of *Solanum virginianum.*

These findings show that the *Solanum virginianum* extract possesses antioxidant activity. DPPH assay revealed that leaf extract had the highest antioxidant activity comparable with Vitamin C (leaf IC_{50}=58.3±0.33µg/mL; Vitamin C=21.7±0.36µg/mL). Total phenolics and Total flavonoid content of leaf extract also highest (1.519 mg gallic acid/100mg and 0.63 mg quercetin/100mg) which attributed to the antioxidant activity of *Solanum virginianum* leaf extract in this study. The leaf extract is a promising candidate for use as natural products based antioxidant for the health of human being. This study suggests that *Solanum virginianum* extract exhibit great potential for antioxidant activity and may be useful for their nutritional and medicinal functions.

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