R.S.A.Sorna Kumar et al /J. Pharm. Sci. & Res. Vol. 8(8), 2016, 725-728

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Anti-Oxidant, Anti-Diabetic,Antimicrobial and Hemolytic Activity of *Solanum Torvum* and *Solanum Trilobatum*

R.S.A.Sorna Kumar*, N.Karthick Raja, M.Vijay, C.Siva Guru Raja.
Department of Biotechnology, P.S.R Engineering College, Sivakasi-626140, Tamil Nadu

Abstract

In the modern time, when pathogens are turning resistive towards drugs, we are inclined to detect a new source that can tackle the situation. One such species which is a potent antimicrobial is Solanum species of Solanacae family. For the present study, *Solanum torvum* and *Solanum trilobatum* were considered. For *Solanum torvum*, maximum crude protein in extract was found out for water extract of fruit(0.73±0.011 mg BSAE) wherein maximum free phenol content was found in water extract of leaf (0.282±0.009 mg GAE). *Solanum trilobatum* gave maximum crude protein content in its water extract of fruit (0.68±0.007 mg BSAE), maximum free phenol was found out in methanolic leaf extract. For hydroxyl free radical scavenging activity, *Solanum torvum* inhibited hydroxyl free radical by 33.72% in its water extract of fruit. This was followed by methanolic extract of leaf, methanolic extract of fruit and water extract of leaf. *Solanum trilobatum* showed a maximum inhibition of 28.72% in its methanolic extract of fruit, which was followed by methanolic extract of leaf, water extract of leaf and water extract of fruit. In-vitro test tube based α–Amylase inhibition activity to determined anti-diabetic activity of samples showed a maximum of 17.4% at pH 3 for water extract of fruit of *Solanum torvum*.

Solanum trilobatum showed a maximum inhibition of 22.72% at pH 3 and 4 followed by 18.92% for water extract of leaf. It also showed a maximum inhibition at pH 3. Hemolytic assay was performed in order to determine hemocyte cleaving activity of the samples, thereby analyzing the cell toxicity of the samples. Methanolic extracts of both the samples showed a slight range of hemolytic activity with methanolic extract of fruit of *Solanum torvum* and *Solanum trilobatum* receiving a hemolytic percentage of 14.37±1.48 and 42.67±2.28 respectively.

Based on the studies done on *Solanum torvum* and *Solanum trilobatum*, both the samples showed promising activity against the parameter studied. Water extract of samples were found to be safe for hemocytes wherein methanolic extracts showed good anti-oxidant, anti-diabetic and anti-microbial activity.

Keywords: Solanacae, S.torvum, S.trilobatum, hemolysis, antioxidant, Anti-diabetic, anti-microbial

1. INTRODUCTION

In the modern time, when pathogens are turning resistive towards drugs, we are inclined to detect a new source that can tackle the situation. One such species which is a potent antimicrobial is *Solanum* species of *Solanacae* family. For the present study, *Solanum torvum* and *Solanum trilobatum* were considered.

Solanum torvum Sw., commonly known sundaikkai in Tamil, is cultivated for its fruits which are used as an essential ingredient in South Indian population's diet. The fruits of *Solanum torvum* are edible and are commonly available in the markets across Tamilnadu either fresh fruits or as dried product. A decoction of fruits is used in treatment of cough ailments and is also considered useful in cases of liver and spleen enlargement [1]. The plant possesses antioxidant, Antimicrobial and Anti-diabetic properties [2][3][4].

Solanum trilobatum is widely used in traditional medicine. Its leaves are mainly used in traditional dishes. It grows to a height of 65 cm and its flowers are violet in color with yellow stamen. Fertilized ovules produce green colored spherical fruits which turn red at maturation. Some of the traditional uses of the plant is to cure asthma, arrest blood vomiting and for reducing blood glucose level. The plant is known to posses excellent antibacterial, antifungal, anti-mitotic, anti-oxidant and anti-tumourous activity [5][6]. The present study utilizes organically grown plant samples whose leaves and ripen fruits for the study of their antioxidant, antibacterial and antitumor activity.

2. MATERIALS AND METHODS

Plant collection and authentication

Seeds of *Solanum torvum* and *Solanum trilobatum* were procured from local market and were grown organically in the garden area of P.S.R Engineering College. Leaves and mature fruits were collected during dawn and were shade dried, powdered and stored in air tight containers for further use.

Plant preparation and extraction

20g of shade dried plant sample powder was extracted with distilled methanol in soxhlet apparatus followed by evaporation of solvent by rotary evaporator to procure methanolic extract.

20g of shade dried plant sample powder was extracted using water by cold maceration method, lyophilized and was used for further studies.

100mg of extract was dissolved in 100ml of their respective solvents and was used as stock solution for further studies.

Total crude protein content

The total crude protein was estimated using Lowry's method. Different dilutions of the extracts were added to 2ml of Lowry's reagent. It was incubated for 10 minutes at room temperature. 0.2ml of Folin-Ciocalteau solution was added to it and incubated for 30 minutes. The absorbance was then taken at 660nm. A plot of Absorbance against Protein concentration was made to get a standard calibration curve. From the curve, the amount of total protein was estimated.

Determination of antioxidant activity of the extracts

Determination of total phenol content

Total free phenolics were estimated using Folin-Ciocalteau reagent [7]. Appropriate dilution of the extracts were oxidized with 2.5 mL of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45°C and the absorbance was measured at 725nm in the UV-Visible spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Hydroxyl free radical scavenging assay

The bioassay was performed according to a previously described procedure [8]. 1.5 mL of different extracts was mixed with 0.02 mL of 30% of H_2O_2 solution. Absorbance was read at 530nm at different times (5–60 min). Decreased absorbance of the reaction mixture indicated increased in scavenging ability. The percentage of inhibition of H_2O_2 radical is calculated using the following equation:

% Inhibition of H_2O_2

$= ((Abs_{control} - Abs_{extract}) / Abs_{control}) * 100$

Where, $Abs_{control}$ is the absorbance of the control (without H_2O_2) and $Abs_{extract}$ the absorbance in the presence of the extracts, then the time required to inhibit 50% (IT50) of H_2O_2 radical was determined.

α-Amylase inhibition assay

This assay was performed in accordance with a method prescribed by Ou S, Kwok K, Li Y and Fu L [9]. Appropriate dilutions of the extracts and 0.5ml of 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl) containing α -amylase (EC 3.2.1.1) (0.5 mg/mL) were incubated at 25°C for 30 minutes. Then, 0.5 mL of 1% starch solution in 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl) was added to the reacting mixture. Thereafter, the reaction mixture was incubated at 25 °C for 10 min and stopped with 1.0 mL of dinitrosalicylic acid (DNSA). The mixture was then incubated in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water, and absorbance measured at 540 nm in a UV-Visible spectrophotometer. Then, the α-amylase inhibitory activity was calculated as percentage inhibition.

% Inhibition

$=[(Abs_{Control} - Abs_{Samples})/Abs_{Control}] * 100$

Antimicrobial assay

Antibacterial assay for the extracts were done by Agar well Diffusion Method [10][11]. 8 hour old cultures were swabbed on nutrient agar plates and using sterile borer, wells of 3 mm diameter and about 2 cm apart were made on each plates. About 100 µl of the plant extract was added into the wells was incubated at 37°C for 24 hours. The development of the zone was noticed, whose diameter was measured. The experiment was repeated thrice to confirm the accuracy of the result.

Hemolytic activity:

5ml of blood was collected from a healthy volunteer at P.S.R Engineering College Clinic and was centrifuged at 2000 rpm for 2 minutes. The supernatant was discarded and pellet was suspended in 5ml of PBS. The supernatant thus obtained was discarded and RBC pellet was used for further study.

Well diffusion method

Hemolytic activity was performed in accordance with the method extracted by R.S.A.Sorna Kumar *et al*.,[12] 1% Agarose solution was prepared using PBS at pH 7.5. The solution was boiled and cooled to 50ºC. To this 0.25ml of egg Yolk and 0.25ml of RBC was added and the solutions was poured into Petri-plates. Using sterile borer, wells of 3 mm diameter and about 2 cm apart were made on each plates. 200µl of plant extracts were poured into these well and the plates were incubated at 37ºC overnight. The zones formed were measured to determine the hemolytic activity.

Phospholipase activity assay

0.25ml of RBC was suspended in 10 ml of PBS at pH 7. This stock was used for study of hemolytic activity. 1ml of the stock was taken and 200µl of extract was added and incubated for 30 minutes at 37ºC. The solution was then centrifuged at 2000rpm for 5 minutes. 0.5ml of supernatant was taken and 1ml of PBS was added. Absorbance was taken at 540nm. 200µl of 30% Triton was used as control.

% hemolytic Activity

= (Absorbance of sample/ Absorbance of Control)

3. RESULT AND DISCUSSION

The experiment studied the total crude protein and total free phenols present in different extracts of *Solanum torvum* and *Solanum trilobatum* and found the values are tabulated in table 1.

	Total Crude protein (Mg BSA Equivalent)	**Total free Phenol** (Mg Gallic acid Equivalent)
Solanum torvum		
Leaf (methanolic)	0.48±0.023	0.132±0.013
Leaf (water)	0.56±0.008	**0.282±0.009**
Fruit (methanolic)	0.63±0.005	0.172±0.004
Fruit (water)	**0.73±0.011**	0.082±0.12
Solanum trilobatum		
Leaf (methanolic)	0.58±0.014	**0.232±0.011**
Leaf (water)	0.64±0.007	0.181±0.008
Fruit (methanolic)	0.49±0.012	0.177±0.004
Fruit (water)	**0.68±0.007**	0.112±0.015

Table 1: Total Crude protein and total free phenols

For *Solanum torvum*, maximum crude protein in extract was found out for water extract of fruit(0.73±0.011 mg BSAE) wherein maximum free phenol content was found in water extract of leaf (0.282±0.009 mg GAE). *Solanum trilobatum* gave maximum crude protein content in its water extract of fruit (0.68±0.007 mg BSAE), maximum free phenol was found out in methanolic leaf extract.

For hydroxyl free radical scavenging activity, *Solanum torvum* inhibited hydroxyl free radical by 33.72% in its water extract of fruit. This was followed by methanolic

extract of leaf, methanolic extract of fruit and water extract of leaf. *Solanum trilobatum* showed a maximum inhibition of 28.72% in its methanolic extract of fruit, which was followed by methanolic extract of leaf, water extract of leaf and water extract of fruit.

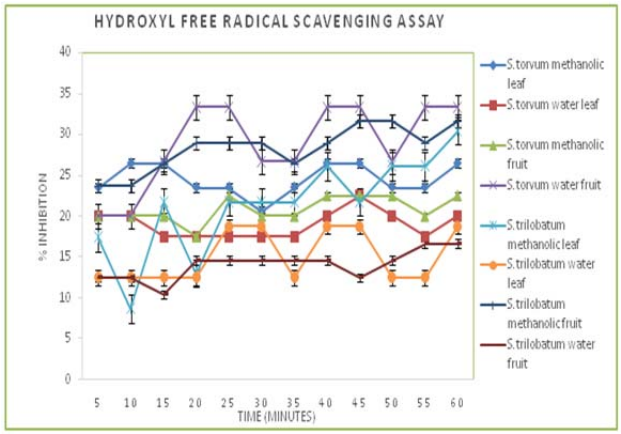

Figure 1: Hydroxyl Free Radical Scavenging Assay

In-vitro test tube based α–Amylase inhibition activity to determined anti-diabetic activity of samples showed a maximum of 17.4% at pH 3 for water extract of fruit of *Solanum torvum*. The maximum inhibition was obtained at pH 3 for *Solanum torvum*. *Solanum trilobatum* showed a maximum inhibition of 22.72% at pH 3 and 4 followed by 18.92% for water extract of leaf. It also showed a maximum inhibition at pH 3.

While studying antibacterial activity of sample by well diffusion method, *Solanum torvum*'s methanolic as well as water extract of leaf gave maximum zone of 1.4±0.02 cm dia and 1±0.012 cm dia against *Bacillus cereus*. Methanolic extract of its fruit showed a maximum zone of 1±0.012 cm dia against *Escherichia coli*, wherein water extract of fruit gave maximum inhibition against *Bacillus cereus* (0.7±0.008 cm dia).

Solanum trilobatum methanolic extract of leaf and fruit were able to inhibit the growth of microorganisms with a maximum zone obtained against *Bacillus cereus* (1.2±0.02 and 0.8±0.01 cm dia respectively). Water extract of its leaf gave a maximum zone of 1±0.01 against *Escherichia coli* and its water extract of fruit against *Pseudomonas fluroscence*(0.6±0.01 cm dia).

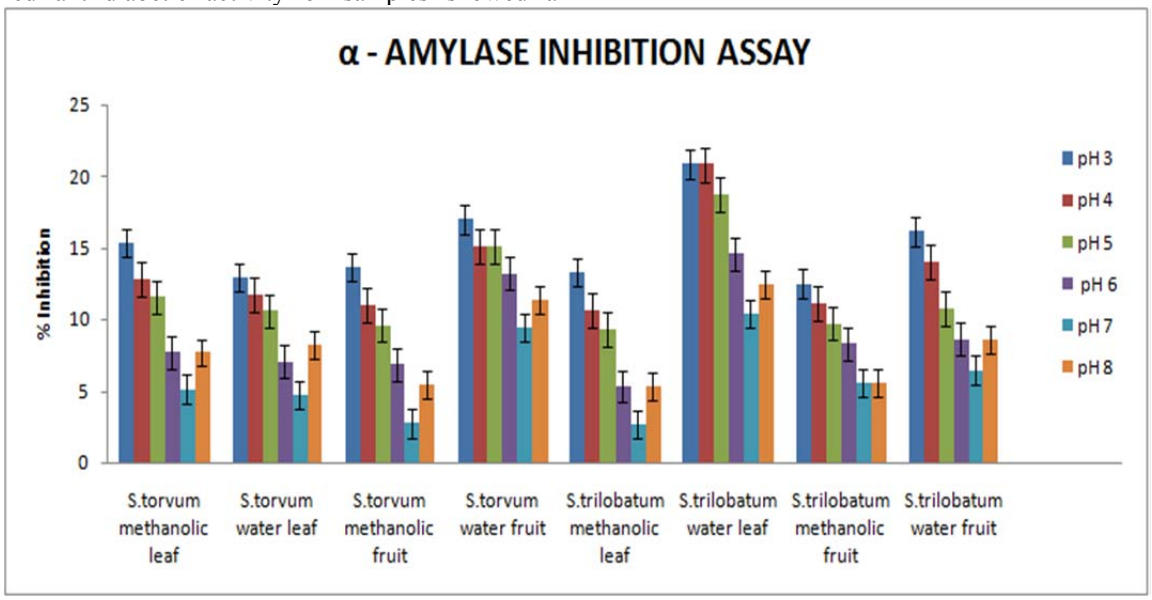

Figure 2: α–Amylase Inhibition Activity

Table 2 Antimicrobial Activity

Microrganism	Diameter of Zone							
	Solanum torvum				***Solanum trilobatum***			
	Leaf Methanol	Leaf Water	Fruit Methanol	Fruit Water	Leaf Methanol	Leaf Water	Fruit Methanol	Fruit Water
E. coli	1.2± 0.02	0.7± 0.1	**1± 0.012**	0.6± 0.01	1± 0.01	**1± 0.01**	0.7± 0.01	0.5± 0.007
B. cereus	**1.4± 0.02**	**1± 0.012**	0.8± 0.01	**0.7±0.008**	**1.2± 0.02**	0.5± 0.01	**0.8± 0.01**	0.2± 0.005
P.fluroscence	1± 0.02	0.7± 0.1	0.8± 0.009	0.3± 0.008	0.8± 0.01	0.3± 0.006	0.4± 0.004	**0.6± 0.01**
P. aeruginosa	0.9± 0.01	0.5± 0.009	0.5± 0.01	0.4± 0.012	0.9± 0.01	0.6± 0.01	0.3± 0.006	0.1± 0.001

Table 3: Hemolytic Activity

	% Hemolysis	**Radius of zone** (mm Dia)
Solanum torvum		
Leaf (methanolic)	11.43± 1.67	0.1± 0.01
Leaf (water)	4.72± 2.67	-
Fruit (methanolic)	14.37± 1.48	0.1± 0.03
Fruit (water)	3.28± 2.85	-
Solanum trilobatum		
Leaf (methanolic)	34.28± 1.82	0.3± 0.05
Leaf (water)	16.32± 3.64	0.1± 0.04
Fruit (methanolic)	42.67± 2.28	0.4± 0.06
Fruit (water)	23.81± 1.83	0.2± 0.03

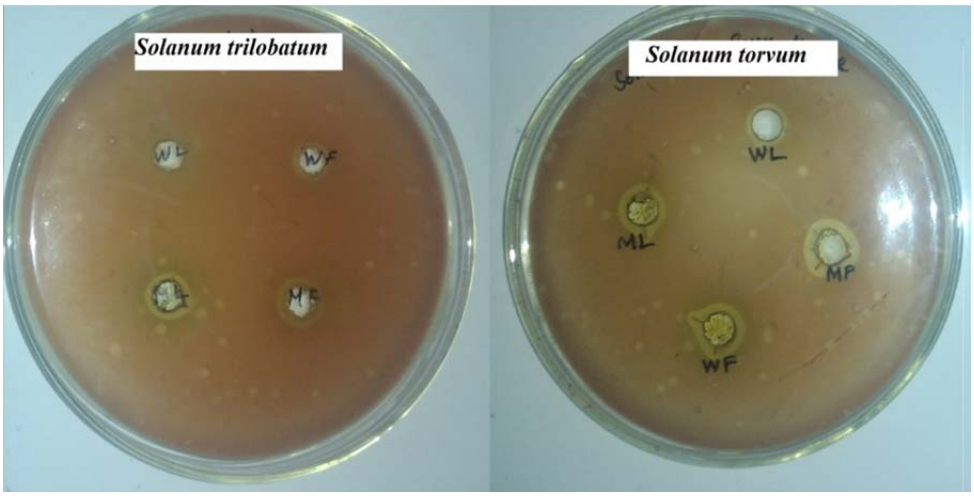

Figure 3: Hemolytic Activity

Hemolytic assay was performed in order to determine hemocyte cleaving activity of the samples, thereby analyzing the cell toxicity of the samples. Methanolic extracts of both the samples showed a slight range of hemolytic activity with methanolic extract of fruit of *Solanum torvum* and *Solanum trilobatum* receiving a hemolytic percentange of 14.37±1.48 and 42.67±2.28 respectively.

4. CONCLUSION

Based on the studies done to study the anti-oxidant, anti-diabetic, anti-microbial and hemolytic activity of *Solanum torvum* and *Solanum trilobatum*, both the samples showed promising activity against the parameter studied. Water extract of samples were found to be safe for hemocytes wherein methanolic extracts showed good anti-oxidant, anti-diabetic and anti-microbial activity.

REFERENCES

1. Siemonsma J, Piluek K, Plant Resources of South-East Asia 8 (PROSEA), Bogor, Indonesia, 1994, pp. 412.
2. Sivapriya M and Srinivas L, Isolation and purification of a novel antioxidant protein from the water extract of Sundakai (Solanum torvum) seeds, Food Chemistry, 104: 510 - 517 (2007).
3. Ajaiyeoba EO, Comparative phytochemical and antimicrobial studies of Solanum macrocarpum and Solanum torvum leaves, Fitoterapia, 70: 184 - 186, (1999).
4. Gandhi GR, Ignacimuthu S, Paulraj MG and Sasikumar P, Antihyperglycemic activity and antidiabetic effect of methyl caffeate isolated from Solanum torvum Swartz. Fruit in streptozotocin induced diabetic rats, Eur J Pharmacol, 30: (23), 623 - 31, (2011).
5. Shahjahan M, Sabitha KE, Mallika Devi R, Shyamala CS (2004). Effect of medicinal plants on tumourogenesis. Ind. J. Med. Res. 123 (5-8): 23-27.
6. Shahjahan M, Vani G, Shyamaladevi CS (2005).Effect of Solanum trilobatum on the antioxidant status during diethyl nitrosamine induced Sofowara A (1993). Medical Plants and Tropical Medicine in Africa. Spectrum Books LTD., Ibandan, Nigeria, pp 289.
7. Singleton, V. L., & Rossi, J. A. ,1965, Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144–158.
8. Axelrod, B., Cheesbrough, T. M., & Laakso, S. ,1981, Lipoxygenase from soybeans. Methods in Enzymology, 71, 441–451.
9. Ou S, Kwok K, Li Y and Fu L.2001, In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. J Agric Food Chem;49:1026-9.
10. Pavitra P.S. Janani V.S., Charumathi K.H., Indumathy R., Sirisha Potala and Rama S. Verma (2012). Antibacterial activity of plants used in indian herbal medicine. *International journal of green Pharmacy*, 23-28.
11. Ajaiyeoba EO, Okogun J (1996).Anthelmintic activity of a root extract of Ritchica capparoides var. longipedicellataPhytother. Res. 10: 436-437.
12. R.S.A.Sorna Kumar, Ajit Vincent Joshua, M.Sangeetha, D.Thilakavathy, Sridevi Gnanaih. Isolation, Purification and Characterization of active compound from Andrographis paniculata.L and testing its antivenom and cytotoxic activity by in-vitro and in-vivo studies. Inj.j.res.Ayurved pharma.2014;5(2):163-168