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Research Article

Evaluation of the Antioxidant, Antimicrobial, Antidiabetic and Hemolytic Activity of Organically Grown *Solanum nigrum* and *Solanum xanthocarpum*

R S A Sorna Kumar*, R J Hariprasanth, M Prem Siddharth, M Gobinath, C Rajukutty

[1]*Department of Biotechnology, P.S.R. Engineering College, Sivakasi-626140, Tamil Nadu*

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ABSTRACT

Medicinal plants have been recognized and used throughout human history. Plants produce many chemical compounds that are for biological functions, including antioxidant, anti-diabetic, antimicrobial and hemolytic activities. In this present study, it was found that the leaf and fruit extracts of *S. nigrum* and *S. xanthocarpum* actively inhibited the growth of the microbes, had minimum hemolytic property, proficient anti-diabetic and anti-oxidant property. For *S. nigrum,* water and methanolic extract of fruit gave the maximum concentration (0.73±0.011 mg BSAE and 0.172±0.004 mg GAE) of total crude protein and total free phenol. *S. xanthocarpum*'s methanolic leaf extract gave the maximum free phenol concentration (0.282±0.011 mg GAE), and water extract of fruit gave the maximum concentration of crude protein (0.68±0.007 mg BSAE). In hydroxyl free radical scavenging assay, methanolic fruit extraction of *S. nigram* gave the maximum inhibition of 33.24±1.72 %. Wherein *s. xanthocarpum*'s water extract of fruit gave the maximum inhibition of 30.72±2.32%. In α-amylase inhibition method, methanolic fruit extract of *S. nigrum* gave a maximum inhibition of 16.28±1.32% at pH 3. *S. xanthocarpum'*s water extract of leaf gave maximum inhibition of 19.21±2.72% at pH 4. Antimicrobial activity by well diffusion method, *S. nigrum*'s methanolic extract of leaf and fruit as well as water extract of leaf showed a maximum zone against *E.coli* and methanolic and aqueous leaf extract of *S. xanthocarpum* showed maximum inhibition in growth of *E.coli*. Remaining organisms were gave mimimum zone of inhibition in all other extracts.

And in hemolytic activity, methonlic extract of leaf and fruit of *S. nigrum* showed higher percentage of hemolysis when compare to its aqueous extract and hemolysis was minute. Methanolic extract of leave and fruit of *S. xanthocarpum* gave 10.48±2.14 and 24.95± 4.29 respectively which is high when compared to the aqueous extract. Methanolic extract *S. xanthocarpum* possess good hemolytic activity.

Keywords: *Solanacae, Solanum nigrum, Solanum Xanthocarpum*, Hemolysis, Amylase inhibition

INTRODUCTION

Plants used in traditional medicine are considered to possess a variety of properties ranging from treating of fever to curing epilepsy. These properties have been tested and passed on to next generation. Two very commonly used medicinal plants are *Solanumnigrum*and *Solanum xanthocarpum* which are also used in daily diet as vegetables. *Solanum nigrum* is commonly found growing as a weed in many parts of Tamil nadu. Its leaves and fruits are mainly used in preparation of traditional dishes. It grows to a height of 45 cm and its flowers are white in colour. Fertilised ovules produce green coloured spherical fruits which turn black at maturation. Presence of various phytochemicals has also been reported in this species[1].

Solanum xanthocarpum is a well-known traditional medicinal plant belonging to the family *solanaceae*. It is popularly known as kandankatthri in Tamil. *S. xanthocarpum* is nontoxic and is safe for human use[2]. The whole plant has many applications like the treatment of dental caries, inflammations, leprosy, skin diseases, cough fever, hemorrhoids and epilepsy. The plant also has anthelmintic, anti-inflammatory, digestive, emmenagogue and aphrodisiac properties[3]. The fruit paste when applied externally to the affected area is known to treat pimples and swellings[4]. The present study aims at analyzing the various properties of organically grown plant samples such as anti-oxidant, anti-diabetic and anti-microbial properties against potent microbial pathogens.

MATERIALS AND METHODS

Plant collection and authentication

Seeds of *Solanumnigrum*and, *Solanum xanthocarpum* were procured from local market and were grown organically in the garden area of PSR 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

*Author for Correspondence:

Table 1: Total Crude protein and total free phenols

	Total Crude protein (Mg BSA Equivalent)	Total free Phenol (Mg Gallic acid Equivalent)
Solanumnigrum		
Leaf (methanolic)	0.48±0.023	0.167±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
SolanumXanthocarpum		
Leaf (methanolic)	0.58±0.014	0.282±0.011
Leaf (water)	0.64±0.007	0.74±0.008
Fruit (methanolic)	0.49±0.012	0.184±0.004
Fruit (water)	0.68±0.007	0.231±0.015

Table 2: Antimicrobial Activity

Microrganism	Diameter of Zone (cm)							
	Solanum nigrum				*Solanum xanthocarpum*			
	Leaf Methanol	Leaf Water	Fruit Methanol	Fruit Water	Leaf Methanol	Leaf Water	Fruit Methanol	Fruit Water
E. Coli	1.0± 0.02	1.2± 0.1	1.2± 0.012	0.6± 0.01	1.4± 0.04	0.9± 0.07	0.7± 0.01	0.5± 0.007
B. cereus	0.9± 0.06	1± 0.08	0.9± 0.01	0.8± 0.01	1.1± 0.02	0.7± 0.01	0.9± 0.03	0.2± 0.005
P.flurosce nce	0.7± 0.02	0.6± 0.1	0.7± 0.009	0.2± 0.008	0.7± 0.03	0.4± 0.006	0.4± 0.004	0.6± 0.01
P. aeruginos a	0.9± 0.12	0.8± 0.09	0.8± 0.01	0.3± 0.008	0.9± 0.01	0.7± 0.01	0.8± 0.006	1.2± 0.01

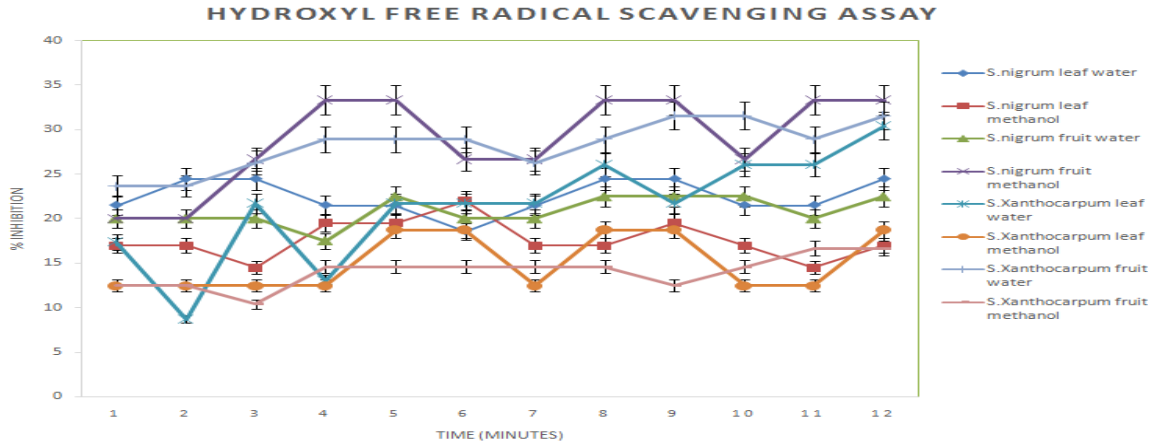

Figure 1: Hydroxyl free radical Scavenging Assay

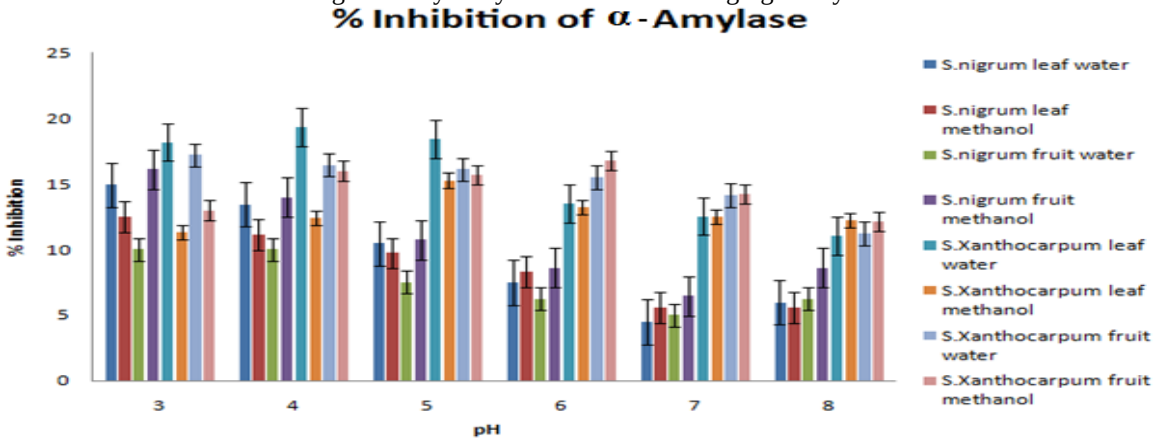

Figure 2: Amylase Inhibition Assay

Table 3: Hemolytic Activit

	% Hemolysis	Diameter of zone (cm)
Solanum nigrum		
Leaf (methanolic)	2.32± 0.92	-
Leaf (water)	1.34± 1.04	-
Fruit (methanolic)	3.07± 0.92	-
Fruit (water)	1.03± 1.12	
S. xanthocarpum		
Leaf (methanolic)	10.48± 2.14	0.1± 0.05
Leaf (water)	5.23± 0.74	-
Fruit (methanolic)	24.95± 4.29	0.3± 0.02
Fruit (water)	7.28±1.72	-

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 Folins-Ciocalteau solution was the 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-Ciocalteu reagent[5]. 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 725 nm 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[6]. 1.5 mL of different extracts was mixed with 0.02 mL of 30% of H_2O_2 solution. Absorbance was read at 530 nm 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% (IT_{50}) of H_2O_2radical 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[7]. 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[8,9]. 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 2000rpm 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

It was done by a method described by R.S.A. Sorna Kumar *et al.*,[10]. 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 was 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.

RESULTS AND DISCUSSION

The experiment studied the total crude protein and total free phenols present in different extracts of *Solanumnigrum* and *Solanum xanthocarpum* and found the values are tabulated in table 1. Total crude protein and free phenolic content of sample were studied in order to estimate the anti-oxidant activity. For *S. nigrum* water extract of fruit and its methanolic extract gave the maximum concentration 0.73±0.011 mg BSAE and 0.172±0.004mg GAE for total crude protein and total free phenol respectively. *S. xanthocarpum*'s methanolic leaf extract gave the maximum free phenol concentration of

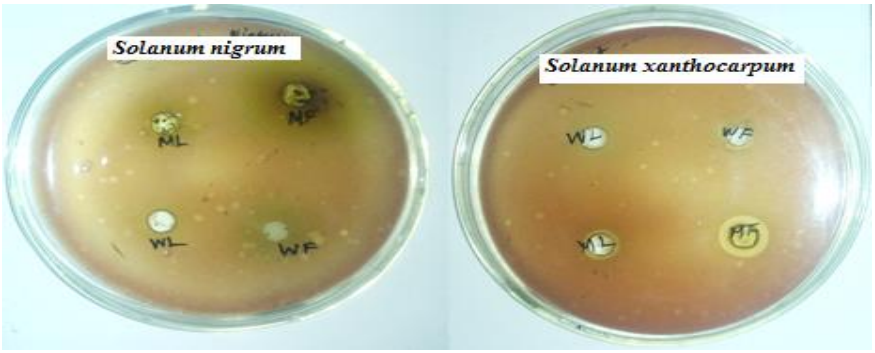

Figure 3: Hemolytic Assay y well diffusion method.

0.282±0.011 mg GAE where in the water extract of fruit gave the maximum concentration of crude protein (0.68±0.007 mg BSAE). Hydroxyl free radical scavenging assay was performed in order to the anti-oxidant activities of samples under consideration. Methanolic fruit extraction of *S. nigram* gave the maximum inhibition of 33.24±1.72 % followed by water extract of leaf, methanolic extract of leaf and water extract of fruit. Wherein water extract of fruit of *s. xanthocarpum* gave the maximum inhibition of 30.72±2.32% followed by water extract of leaf, methanolic leaf extract and methanloic fruit extract. α-Amylase inhibition activity is a test tube based method to study the anti-diabeticproperty of different extract. Methanolic fruit extract of *S. nigrum* gave a maximum inhibition of 16.28±1.324% at pH 3. This was followed by water extract of leaf, methanolic leaf extract and water extract of fruit. The optimum pH for the activity of*s. nigrum* was found to pH 3. *S. xanthocarpum'*s water extract of leaf gave maximum inhibition of 19.21±2.72% at pH 4, this was followed by water extract of fruit at pH 3, methanolic fruit extract at pH 6 and methanolic leaf extract at pH 5. Antimicrobial activity was performed in order to study the inhibition of growth of certain microorganism by well diffusion method. In case of *s. nigrum*methanolic extract ofleaf and fruit as well as water extract of leaf showed a maximum zone against *E.coli* .The water extract of fruit was able inhibit *B.cereus'*s growth when compared to other microbes under study. Methanolic and aqueous leaf extract of *S. xanthocarpum* showed maximum inhibition in growth of *E.coli* where in methanolic fruit extract gave maximum zone against *B. cereus* and aqueous extract of fruit against *P. aeruginosa*. Hemolytic activity was performed in order to study the hemotoxic activity of the samples on hemocytes. Methonlic extract of leaf and fruit of *S. nigrum* showed higher percentage of hemolysis when compare to its aqueous counterpart. But the hemolysis was minute. Methanolic extract of leave and fruit of *S. xanthocarpum* gave 10.48±2.14 and 24.95± 4.29 respectively which is high when compared to the aqueous extract. Methanolic extract *S. xanthocarpum* possess good hemolytic activity which may be the same for cytotoxicity.

CONCLUSION

The study performed in order to evaluate the antioxidant ant diabetic, antimicrobial and hemolytic activity of *S. nigrum*. *S. xanthocarpum* showed considerably positive result. Apart from methanolic extract of *S. xanthocarpum*, all other extract did not show hemotoxic effect but all the extract under study showed good anti-oxidant anti-diabetic, anti-microbial activity.

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