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Research Article

IN VITRO ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND TOTAL FLAVANOID CONTENT OF DIFFERENT EXTRACTS OF *SOLANUM XANTHOCARPUM* BERRIES

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ABSTRACT

In this present study, the antioxidant activity of various extracts of berries of *Solanum xanthocarpum* was studied using *in vitro* assay. The antioxidant activity of *S. xanthocarpum* was evaluated by using the free radical scavenging activity assay (DPPH method). Total phenolic content was determined by using gallic acid as a standard and the Total flavanoid content was measured by taking quercitin as a standard. The results of the study shows that the ethanolic extract of berries shows maximum free radical scavenging properties and a correlation exists between the Antioxidant potential and Phenolic content.

Keywords: *Solanum xanthocarpum,* Free radical scavenging activity, DPPH, Total Phenolic, Total Flavanoid.

INTRODUCTION

Oxygen is essential for the survival of all living organism on this earth. During this process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalentently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals[1,2]. Reactive Oxygen Species (ROS) react easily with these free radicals to become radicals themselves[3], which results in numerous diseases and disorders[4,5] such as cancer[6], cardiovascular disease[7], neural disorders[8], Alzheimer's disease[9], mild congnitive impairment[10], Parkinsons disease[11], alcohol induced liverdisease[12], ulcerative colitis[13], ageing[14], atherosclerosis[15]. In vivo, some of these ROS also play an important role in cell metabolism including energy production, phagocytosis and intercellular signaling[16]. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA stand breaking[17]. Over the last few decades**,** there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have few or no side effects for use in preventive medicine and in the food industry.

Solanum xanthocarpum (Family:Solanaceae) commonly known as Indian night shade or yellow berried night shade plant. It is commonly growing perennial herbaceous weed with bright green leaves and zigzag stem, mostly found in the arid region[18]. The flowers are purple in color. The flowers bloom in Oct – March. The berries are yellow with many seeds. Different parts of this plant are used in the treatment of the diseases like fever, asthma, tuberculosis, kidney disorder, cough, constipation, tooth ache, sore throat, rheumatism and gonorrhea [19]. It is one of the members of the dashamula (ten roots) of the Ayurveda [20]. It is found that the plant has solasodine in its different parts, which is responsible for its medicinal value [21]. Phytochemical screening of the plant shows the presence of alkaloids, glycosides, flavonoids, tannins and triterpenoids. So far the antioxidant property of the Solanum xanthocarpum berries was not reported. So the present study was assessed to evaluate the Total Phenolic Content, Total Flavanoid Content and In vitro Antioxidant activity of different extracts of Solanum Xanthocarpum.

MATERIAL AND METHODS

Dried whole plant of *S. xanthocarpum* **(**Solanaceae) was procured from supplier in New Delhi, and verified by Dr. H. B. Singh, Head, National Institute of Science Communication And Information Resources (Council of Scientific and Industrial Research) 14 against a voucher specimen number NISCAIR/RHMD/Consult/-2010-11/1602/202.

All the chemicals and reagents used were obtained from Qualigens Fine Chemicals, Mumbai, Merck Specialties Private Ltd., and were of analytical reagent (AR) grade. Double beam UV-Visible spectrophotometer (Perkin Elmer Lambda 25 UV/VIS Spectrometer) was used for determination of Total phenolic, Total flavanoid and Antioxidant activity.

Spectrophotometric Procedure

Determination of Total phenolic content

Total phenol estimation can be carried out with Folin-Ciocalteu reagent (FCR)[22].

Reagents

50% Methanol, Folin-Ciocalteu reagent (FCR), 20% Sodium carbonate, Standard (5 mg gallic acid in 5 ml of 50% methanol)

Method

5 mg of the sample was weighed and dissolved in 1 ml of 50% methanol using a vortex mixer (Touch Type) followed by adding to it 4 ml of 50% methanol and finally mixing through sonication to prepare a sample of concentration 1 mg/ml. 0.5 ml of this solution was pipette out in a test tube to which was added 3.5 ml of distilled water followed by addition of 0.25 ml of Folin-Ciocalteu reagent (FCR). This was left for incubation for 1-8 minutes at room temperature. Lastly, to this 0.75 ml of 20% sodium carbonate solution was added and the final sample solution in the test tube was left to incubate for 2 hours. The sample was prepared in duplicate. Finally, the absorbance was measured at 765 nm against a reagent blank. Same procedure was repeated for all the extracts of *S. xanthocarpum*. The standard curve was obtained using gallic acid monohydrate.The Total phenol content will be expressed as gallic acid equivalent to % w/w of the extracts [23].

Determination of Total flavonoid content

The Total flavanoid content of the extracts can be determined by Aluminium chloride colorimetric method [24].

Reagents

80% ethanol, 10% Aluminium chloride (in distilled water), 1M potassium acetate aqueous solution, Methanol, Standard (1 mg quercetin in 10 ml of 80% ethanol)

Method

10 mg of the sample was weighed and dissolved in 1 ml of 80% ethanol using a vortex mixer (Touch Type) followed by adding to it 9 ml of 80% ethanol and finally mixing through sonication to prepare a sample of concentration 1 mg/ml. 0.5 ml of this solution was pipette out in a test tube to which 1.5 ml of methanol, 0.1 ml of 10%

aluminium chloride, 0.1 ml of 1M potassium acetate aqueous solution and 2.8 ml of distilled water was added. A yellow color indicates the presence of flavonoids. The final sample solution in the test tube was left to incubate for 30 minutes at room temperature. The sample was prepared in duplicate. Finally, the absorbance was measured at 415 nm against a reagent blank. Same procedure was repeated for all the extracts of *S. xanthocarpum*. The standard curve was obtained using quercetin using solution in the range of 1-10 µg/ml [25].The results will be expressed as mg quercetin/g dry weight by comparison with quercetin standard curve, which was made under the same conditions.

Antioxidant activity by DPPH free radical scavenging assay

The antioxidant activity of the extracts and standard compound (ascorbic acid) can be evaluated on the basis of radical scavenging effect of the stable DPPH free radical. About 10 mg of the sample was weighed and dissolved in 1 ml of methanol using a vortex mixer (Touch Type) followed by adding to it 19 ml of methanol and finally mixing through sonication to prepare a sample of concentration 0.5 mg/ml. DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml of methanol. Various dilutions of the sample were prepared with methanol resulting in concentrations 5oµg/ml, 100 µg/ml, 150µg/ml, 200 µg/ml, 250 µg/ml, 300 µg/ml and 350 µg/ml. To each dilution 2 ml of the prepared DPPH solution in methanol was added. A control was also prepared simultaneously consisting of 2 ml methanol and 2ml DPPH solution. The prepared dilutions were then left for colour development in the dark for 20 minutes. Finally, the absorbance was measured at 517 nm against a reagent blank. Same procedure was repeated for all the etracts of *S. xanthocarpum*. The standard curve was obtained using ascorbic acid. A plot of concentration vs. the percentage inhibition of DPPH radical gave the IC_{50}value which is the concentration of sample required to inhibit 50% of DPPH radical [26].

$$\% \text{ inhibition} = (A_{control} - A_{sample}) / A_{control} X\ 100\ \%$$

Total Phenolic Content

RESULTS & DISCUSSION

Determination of Total phenolic and Total flavonoid content by Spectrophotometric Procedure

Total phenolic and Total flavonoid content in the extracts were determined from a regression equation from a calibration curve (y= 0.0975x+ 0.2846, R^2 =0.9944) and (y= 0.0037x+0.0027, R^2 =0/998) respectively (Fig 1 &2). The amount of Total phenolic and Total flavonoid content of methanolic, ethanolic and aqueous extracts are demonstrated in Table 1and 2. Ethanolic extract revealed the highest Total phenolic content and Total flavonoids contents at 9.02mg GAE/L and 36.16mg QE/L respectively. From the literature survey, we know that Total phenolic and Total flavonoid content may contribute directly to antioxidant action. Therefore, ethanolic extract may be the most effective extracting solvents for antioxidants from the berries of *Solanum xanthocarpum*. These results show that the antioxidant activity of extracts of the berries of this plant may be related to their phenolic or flavonoid substrates.

Effects of DPPH scavenging activity

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. The reduction capability of DPPH radical was determined by using absorbance of 517nm, which is induced by antioxidants. The scavenging abilities of various extracts against DPPH radical were concentration-dependent, and increased steadily with time. According to the results IC_{50} value of ascorbic acid was found to be 49µg/ml (Fig 3) while all the extracts show different IC_{50} value at different concentration. Accordingly ethyl acetate extract shows minimum IC_{50} value at 620µg/ml and ethanol extract shows higher IC_{50} value at 160µg/ml and IC_{50} value of chloroform, methanol and aqueous extracts were found to be 380, 250 and 180 µg/ml respectively (Table 3) This shows that all the extracts of *Solanum xanthocarpum* berries possess hydrogen donating capabilities and can act as an antioxidant.

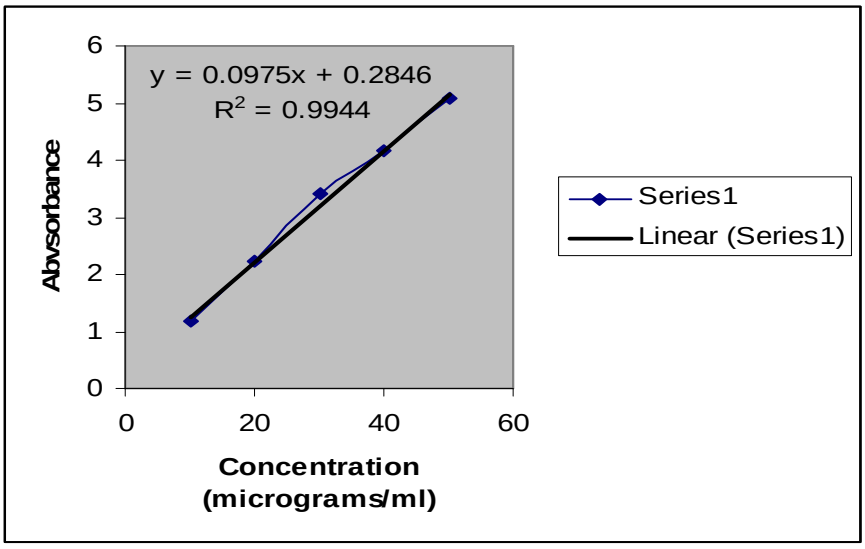

Fig. 1: Standard curve for total phenol estimation with gallic acid

Table 1: Observation Of Total Phenol Content

S. No.	Extract	Total Phenol (mg GAE/L)	Average (mg GAE/L)
1	Aqueous	i. 1.05 ii. 1.22	1.13
2	Ethanolic	i. 8.90 ii. 9.15	9.02
3	Methanolic	i. 2.25 ii. 1.95	2.1

Total Flavanoid Content

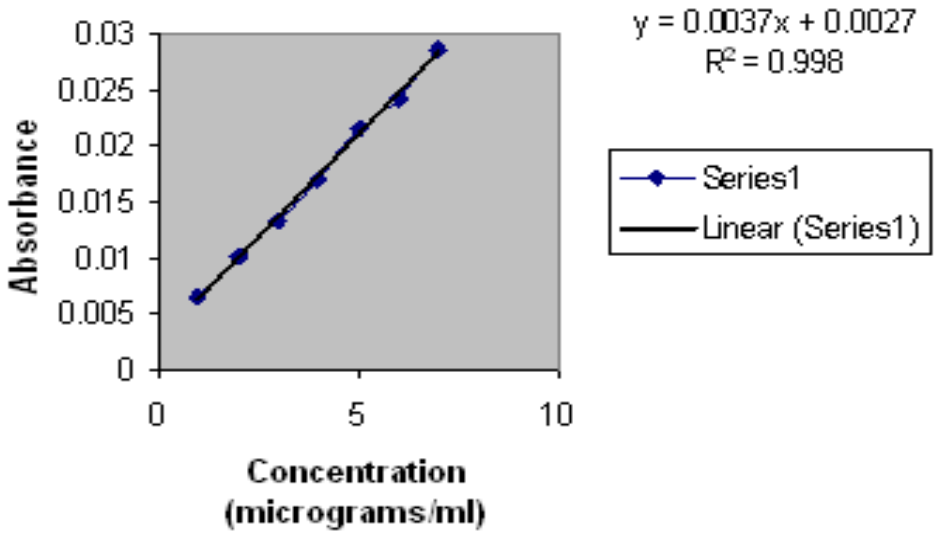

Fig. 2: Standard curve for total flavonoid estimation with quercetin

Table 2: Observation of total flavanoid content

Extract	Total Flavanoid (mg QE/L)	Average (mg QE/L)
Aqueous	i. 14.90	
	ii. 6.24	10.57
Ethanolic	i. 49.05	36.16
	ii. 23.27	
Methanolic	i. 12.40	9.00
	ii. 5.60	

Table 3: Observation of antioxidant activity of different extract of *S. xanthocarpum* Berries

S. No.	Extract	IC_{50}(µG/ML)
1	Ethylacetate	620
2	Chloroform	380
3	Methanol	250
4	Aqueous	180
5	Ethanol	160

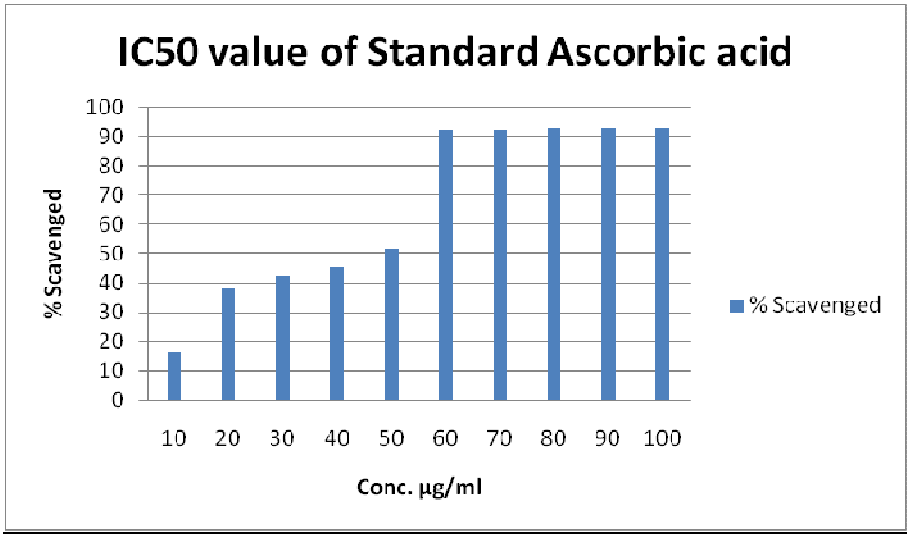

Fig. 3: IC_{50}value of ascorbic acid

(IC_{50}VALUE = 49 µG/ML)

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