Phytochemical Investigation and Pharmacological Evaluation of Solanum xanthocarpum Endowed with their potential Activity

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Abstract: The study was done to assess the *in-vitro* antibacterial potential of various extracts was studied and compared with ciprofloxacin as the standard and shows significant action against E. coli, B. substilis S. aureus, S. pyrogenes, P. aeruginosa, and S. typhi. Anti-fungal potential of the aqueous extract also studied using miconazole as standard and shows significant action against A. niger and C. albicans. Anthelmintic potential of the aqueous and ethanolic extracts was also studied on earthworms, Eudrillus eugeniae using albendazole as standard and shows moderate activity. In the present study in-vitro free radical scavenging activity of whole plant material performed. Various crude extracts of S. xanthocarpum was prepared by successive maceration process using various solvents such as; chloroform, petroleum ether (60-80°), acetone, ethanol and distilled water. Each one extract have been chosen to study the free radical inhibitory activity by DPPH radical scavenging method. The preliminary phytochemical screening of extracts showed that sterols, alkaloids, glycosides, tannins, saponins, phenolic compounds, carbohydrates and proteins were present in the plant. Petroleum ether, chloroform, acetone, ethanol and distilled water extracts showed 52.69, 46.15, 21.08, 52.72 and Journal of Pharmaceutical 44.35 % respectively compared to standard ascorbic acid. Acetone extract showed poor inhibition of DPPH radical compared to standard and other extracts also.

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Chhajed, M Jain, A Gupta, S Dubey, I Shrivastava, AK **Keywords:** S. xanthocarpum, antibacterial, antifungal, DPPH free radical, phytochemical screening.

1. INTRODUCTION

Solanum xanthocarpum Schrad and Wendl is significant remedial plant belong to family Solanaceae, which finds uses in Ayurvedic system of medicine such as respiratory tract infection, asthma, cough, common cold, antipyretic, dyspnea, dropsy, and chest pain.¹⁻³ The roots are used in fever, cough, asthma, inflammation and as diuretic & antiemetic.⁴⁻⁶ Chronic cough in children relieves if the powdered fruit is given with honey. *S. xanthocarpum* fruits juice is used in treatment of sore throats and the burning seeds vapours relieves toothache.⁷ The fruit extract is also showed the antispermatogenic action in rats.⁸⁻⁹ It also been reported that fruits and roots of the plant exhibit larvicidal activity aligned with tracks of malaria and dengue.¹⁰

It is usually called as kanthkari and kateli. It's occurs commonly in waste places, roadsides and along railway lines through out India. It is very spiny, diffused much -branched, perpetual plant attaining height up to 1.2 m. The leaves are light green in color and having purplish hairs on both surfaces, ovate or elliptic, sinuate or sub-pinnatified; spines are 1 cm long, sharp, straight and yellow; flowers violet in lateral cymes; berries green in color with whitish patches when young, yellow and glabrous with greenish white veins, 1.2 to 2 cm in diameter; seeds glabrous; roots are 3-5 cm long and 1.5-6 mm thick, dreary grayish with a spongy fibrous splinter stem, flowers and fruits are bitter and carminative.^{9,11-13}

The growing interest in herb and offer economical uses of plant is part of the movement towards "greener" economics and life styles.¹⁴ Medicinal plants have fascinated concentration of not only medicine professionals of assorted systems, but also the scientific societies belong to diverse disciplines. Herbal medicines, generally consider not detrimental in given set doses, are turning into trendy throughout the world and WHO presently persuade, counsel and endorse enclosure of these remedies in nationwide health care programme.¹⁵ In current time, there has been an enormous awareness in herbal medication for the management of number of diseases.

Presently there has been an augmented interest worldwide to recognize antioxidant composite that are therapeutically strongly effective and have stumpy or lack of adverse effects to be used in prophylactic medication. As plants manufacture considerable quantity of antioxidants to avoid the oxidative stress originated by photons and oxygen, it represents a impending resource of innovative compounds with antioxidant potential.

Bearing in mind the mounting attention in evaluating the antioxidant capability of herbal drugs in this research, we studied the antioxidant properties of *S*. xanthocarpum.¹⁶ Medicinal plants are assured source of drugs. *S xanthocarpum* is a widely available plant in India and very less work has been published so far on antimicrobial viz antibacterial and antifungal, anthelmintic and antioxidant activity. The current research work deals with aforesaid activity of the *S. xanthocarpum* using standard laboratory model.

2. MATERIAL AND METHODS CHEMICAL AND REAGENTS

Ciprofloxacin, miconazole and albendazole was received as a gift sample from IPCA Laboratories, Mumbai, India,. Nutrient agar, Sabourd's agar medium and ascorbic acid were purchased from Loba Chem, Mumbai, India. DPPH was purchased from Merck Lab. Mumbai, India. All other reagents and chemical were analytical grade and used as procured.

2.1 Collection of Plant Material and Preparation of Extract

The whole plant material was collected in month of March 2005 from Chennai and shade dried. The plant material was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai. The taxonomic identification was carried out following Keshavamurthy and Yoganarasimhan (1990) and Gamble(1967).¹⁷⁻¹⁸

Shade dried, powdered plant material was extracted in close vessel by maceration process for 72 h at room temperature using petroleum ether (60- 80°), chloroform, acetone, ethanol and distilled water (contains 0.25% v/v chloroform as preservative). Distillation process under reduced pressure to get solid mass (i.e. completely free from the solvent) was employed to get the extract concentrate. The extractive value was found to be 12.36% (petroleum ether), 11.61% (chloroform), 8.98% (acetone), 8.29% (ethanol) and 9.48% (distilled water) with respect to the dry starting powdered plant material. These extracts were subjected for phytochemical evaluation.

2.2 Micro-organisms

Six clinical strains *Escherichia coli, Bacillus substilis Staphylococcus aureus, Streptococcus pyrogenes, Pseudomonas aeruginosa,* and *Salmonella typhi* were used for evaluating the antibacterial potential against standard ciprofloxacin (10 μ g/mL). Two fungal strains *Aspergillus niger* and *Candida albicans* were used for antifungal activity determination using Miconazole (10 μ g/mL). Earthworms- *Eudrillus eugeniae* were used for assessment of anthelmintic activity using standard albendazole (25, 50, 100 mg/mL). All the microorganisms were obtained from Rajiv Gandhi Vikas Biotechnology Centre, LIT Campus, RTM Nagpur University, Nagpur. Earthworms

Chhajed, M Jain, A Gupta, S Dubey, I Shrivastava, AK were obtained from Horticulture Department, Panjabrao Deshmukh Krishi Vidhyapith, Nagpur. The cultures were checked for their purity by conformist cultural, morphological and biochemical methods. The obtained bacterial and fungal cultures were upholded and hoard in nutrient agar and Sabouraud's agar medium respectively at 4° C.

2.3 Antimicrobial Activity

The agar cup-plate method was employed to assess the antimicrobial activity of the prepared extracts. Nutrient agar and Sabourd's agar medium was employed as growth medium for bacterial and fungal strain respectively. Control experiment was performed in the identical condition by employing ciprofloxacin (10 μ g/mL) and miconazole (10 μ g/mL) as standard for antibacterial and antifungal activity respectively. The inoculated bacterial and fungal strains were incubated at 37 ± 2° C for 24 hrs and 27 ± 2° C for 48 hrs respectively.¹⁹ The evaluation of activity against microbial growth was based on the measurement of diameter of zone of inhibition formed by the plant material extract in dimethyl sulphoxide (DMSO) and standard drugs also.

2.4 Anthelmintic Activity

The anthelmintic activity of *S. xanthocarpum* was determined by the method of Gunasekaran (2006).²⁰ The anthelmintic potential was assessed on adult Indian earthworm - *Eudrillus eugeniae*. Three groups of just about equal size worm comprisinging of six worms in every cluster were relieved in 50 mL of preferred formulation. Further all group was treated with one of the following- control vehicle (1% gum acacia in normal saline), ethanolic and aqueous extracts of diverse concentrations (25, 50, 100 mg/mL) and albendazole (25, 50, 100 mg/mL)

2.5 DPPH Radical Scavenging Activity

Free radical wiping potential of every one extracts were assessed for their *in vitro* free radical scavenging activities by DPPH radical assay procedure.²¹

A process foundation on the reduction of the coloured DPPH radical solution in methanol has been used to assess the free radical scavenging activity. DPPH reagent 50 μ L (conc 2 mg/mL) have been added to test tubes having 3 mL of methyl alcohol and the preliminary absorbance measured on UV-Visible spectrophotometer. To the aforesaid test tubes, different test solutions of extract in methanol (1 mg/mL) have been added (10-50 μ L). Further ascorbic acid (0.5 mg/mL) have also been added in the range of 10-50 μ L and set aside. Following 20 minutes, absorbance has been measured at 516 nm. The experimental procedures were carried out in triplicate. The

percentage of reduction in absorbance was calculated from the initial and final absorbance of each tested solution.²¹⁻²² Percentage of DPPH radical scavenging was assessed using following formula,

% DPPH Radical Scavenging = $\frac{\text{control} - \text{Test}}{\text{Control}} \times 100$

3. RESULTS

3.1 Phytochemical Investigation

The phytochemical screening of extracts showed the incidence of sterols, alkaloids, glycosides, tannins, saponins, phenolic and flavonoid compounds, carbohydrates and proteins within the plant, amongst them sterols and phenolic compounds may responsible for the antioxidant activities. TLC also reveals that alkaloids, glycosides, sterols, saponin and phenolic compounds are present in *S. xanthocarpum*. The results are reported in table 1.

Table 1: Phytochemical Screening of Solanum xanthocarpum.

Extract	Tannin	Glycoside	Sterols	Alkaloids	Saponin	Reducing sugar
Aqueous	+	+	_	+	+	+
Ethanolic	+	+	+	+	+	+
Acetone	_	+	+	+	+	+
Chloroform	_	+	+	+	_	+
Petroleum ether	_	_	+	+	_	+

3.2 Antimicrobial activity

The diameter of zone of inhibition is shown in Table 2. Activity index²³ was also calculated and reported in the Table 2. The control (DMSO) showed no inhibition of growth while all the extracts were exhibited activity against bacteria and fungus, when compared to ciprofloxacin and miconazole respectively.

3.3 Anthelmintic activity

Anthelmintic activity of ethanolic and aqueous extracts of *S. xanthocarpum* against earthworm - *Eudrillus eugeniae* is shown in Table 3. Ethanolic extract

Jain, A					5			1		
Gupta, S Dubey, I			Diameter of Zone of Inhibition in mma (Activity index) b							
Shrivastava, AK	Extract	Conc	Bacteria						Fungus	
			E. coli	B. substilis	S. aureus	S. pyrogenes	P. aeruginosa	S. typhi	A. niger	C. albicans
	Petroleum ether	100 mg/mL	$17 \pm$.0.76 (0.68)	21 ± 0.90 (0.78)	0.19 ± 0.95 (0.70)	512 ± 1.09 (0.50)	11 ± 0.90 (0.42)	19 ± 0.91 (0.68)	10 ± 0.63 (0.53)	11 ± 1.33 (0.61)
	Chloroform		15 ± 1.4 (0.6)	415 ± 1.32 (0.56)	218 ± 1.34 (0.67)	13 ± 1.32 (0.54)	11 ± 1.24 (0.42)	21 ± 0.74 (0.75)	16 ± 0.88 (0.84)	$15 \pm .093$ (0.83)
	Acetone		16 ± 1.1 (0.64)	318 ± 0.86 (0.67)	521 ± 0.74 (0.78)	15 ± 1.54 (0.63)	12 ± 1.24 (0.46)	$\begin{array}{c} 20.5 \pm \\ 0.88 \\ (0.73) \end{array}$	12 ± 0.95 (0.63)	10 ± 0.96 (0.56)
	Ethanol		20 ± 1.0 (0.8)	124 ± 0.89 (0.89)	0.023 ± 1.12 (0.85)	12 ± 0.99 (0.50)	14 ± 0.94 (0.54)	22 ± 1.51 (0.79)	14 ± 1.44 (0.74)	13 ± 1.02 (0.72)
	Distilled water			716 ± 0.99 (0.59)	0.017 ± 0.76 (0.63)	0.11 ± 0.80 (0.46)	$\begin{array}{c} 10\pm1.08\\(0.38)\end{array}$	23 ± 1.28 (0.82)	314 ± 1.29 (0.74)	14 ± 0.68 (0.78)
	Ciprofloxacin	10 μg/ mL	25 ± 0.9	127 ± 1.44	127 ± 0.69	24 ± 1.53	26 ± 1.32	28 ± 1.17	V NA	NA
	Miconazole	10 μg/ mL	NA	NA	NA	NA	NA	NA	19 ± 0.73	18 ± 1.11
	0	6.1	1.	1		1.		- 14		

Table 2: Antimicrobial Activi	y of Solanum xanthocar	pum Sehrad. & Wendl.
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 a^{a} – an average of three readings, values are expressed in mean \pm SEM.

^b – (Activity index) = Inhibition zone of the extract / Inhibition zone of the standard NA: not applied

shows significant activity over aqueous extract when compared to standard drug albendazole in same concentration.

3.4 Free radical scavenging activity

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Ethanolic extract of *S. xanthocarpum* had showed 50.73% inhibition of DPPH radical, which is 52.72% free radical scavenging activity compared to standard, ascorbic acid. Petroleum ether extract also showed almost similar activity (52.69% compared to standard), while chloroform and aqueous extracts showed 46.15 and 44.35% scavenging activity respectively compared to standard whereas acetone extract showed poor inhibition of DPPH radical (21.08%) compared to standard and other extracts. The results are reported in Table 4. IC₅₀ value for each extract and standard has been calculated and linear regression curve for log value of IC₅₀ is established and revealed in figure I.

	lis - Luarillus eas	zeniae.		Pharmacological
E. (Concentration	Time in minutes f	or paralysis/deatha	Evaluation
Extract/Drug	(mg/mL) —	Paralysis	Death	- of Solanum xanthocarpum
Albendazole	25	20.91 ± 0.50	31.49 ± 0.82	Endowed with their
	50	15.66 ± 1.18	28.74 ± 0.62	potential Activity
	100	12.37 ± 1.37	22.66 ± 0.58	
Ethanolic extract	25	24.86 ± 0.69	37.73 ± 0.86	
	50	20.65 ± 0.96	$33.09.\pm0.97$	
	100	17.38 ± 1.02	28.37 ± 1.36	
Aqueous extract	25	29.87 ± 0.86	45.36 ± 1.07	
	50	26.22 ± 1.03	39.36 ± 0.92	
	100	21.92 ± 1.22	32.13 ± 0.94	

Phytochemical

Investigation and

Table 3: Anthelmintic Activity of Solanum xanthocarpum Sehrad. & Wendl.Against earthworms – *Eudrillus eugeniae*.

 $^{\rm a}$ – an average of six readings, values are expressed in mean \pm SEM. Control worms were alive upto 24 hr. of observation

Table	4:	Free	radical	scavenging	activity	of	extract	of	Solanum
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Compd. No.a	% Inhibition of DPPH radicalb	% Free radical scavenging activityb
Standard ^c	96.23	100
Petroleum ether	50.70	52.66
Chloroform	44.41	46.15
Acetone	20.29	21.08
Ethanol	50.73	52.72
Water	42.68	44.35

 $^{\rm a}$ Concentration of test compounds was 50 mg/mL, concentration of $\,$ standard was 0.5 mg/mL.

 b Activity reported at concentration of 50 μL of DPPH reagent, values are reported as mean $\pm SEM$

° Standard used was ascorbic acid



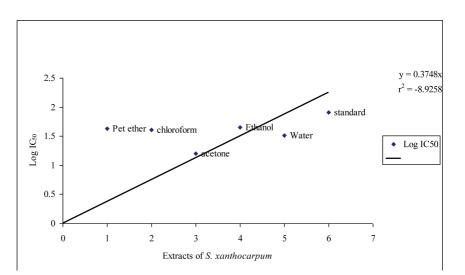


Figure 1: Log IC_{50} and linear regression.

DISCUSSION

Antioxidants put efforts to manage the intensity of free radicals earlier than they do oxidative damage with in body. For example, variety of enzymes in the body, such as superoxide dismutase, work with additional compounds to convert free radicals into undamaging molecules. Certain disorders like Cancer, atherosclerosis, cirrhosis, emphysema, and arthritis have been interrelated in the midst of oxidative damage.²⁴ Vitamin C which having antioxidant property which may have preventive action on cataracts and variety of cancers such as cancers of the mouth, esophagus, stomach, and pancreas.²⁵ In addition it possibly will avoid the oxidation of LDL cholesterol which may be responsible for lesser the threat of heart ailment.

Geronikaki A, *et al.*,²⁶ in 2003 discloses that, the ketonic groups are accountable for scavenging of free radicals. Free Radicals can be defined as atoms or group of atoms which having an unpair electrons and formed by interactions of definite molecules with oxygen. Formerly extremely reactive free radicals produced initiate a chain reaction resembling dominoes. Their major hazard appeared from the damage; they are able to accomplish when they react with significant cellular machinery for instance DNA, or cell membrane. Under the instance cells possibly will work inadequately or die. The body has a security arrangement of antioxidant to avoid free radical damage.²⁷ Antioxidants are capable to furnish free radicals, which turn out to be a companion to unpaired electron of preformed free radical, as a consequence eradicate the menace of gene modifications leading to malignancy.²⁸⁻²⁹

Certain environmental circumstances, life style factors and standard cellular function throughout body possibly will responsible for the free radical production. These molecules are missing an electron, giving them an electric charge. Free radicals attempt to take an electron from, or give an electron to adjacent molecule, generates a newer free radical from the adjacent molecule, which may lead to neutralization of the charge. The recently formed free radicals, in turn, hunting for an additional molecules and give or take an electron, lay down a series reaction that is able to smash up hundreds of molecules. Antioxidants stop the progress of this series reaction. Several antioxidants are solitary act as free radicals. Further antioxidants work aligned with the molecules that are able to produce free radicals, wipe out them earlier than they can commence the domino consequence that show the way to oxidative damage.³⁰

The current research reveals that the *Solanum xanthocarpum* encompass pharmacologically dynamic substances such as alkaloids, glycosides, saponins, phenolic compounds and flavonoids, which are accountable for scavenging properties of free radical. The incidence of alkaloids, phenolic compounds and flavonoids in the alcoholic and petroleum ether extract may be responsible for its antioxidant activity than other extracts. So isolation and further analysis of these extracts may reveal, which compound is responsible for its activity and help in further investigation on the mechanism of antioxidant activity of these compounds. Auxiliary researches are in development in our laboratory to assess the other *in-vitro* models and *in-vivo* antioxidant prospective of the extracts in different animal models. Phytochemical analyses are also obligatory to ascertain the category of components liable for the thearapeutic activity of this remedial plant and to conclude the significance of the ethnobotanical approach for the evaluating of plants as budding resource of bioactive materials.

The phytochemical screening of extracts was done³¹ and found that sterols, alkaloids, glycosides, tannins, saponins and reducing sugar were present in the plant amongst them alkaloids may responsible for these activities.

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