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Phytochemical Investigation and Pharmacological Evaluation for Antimicrobial, Anthelmintic and Antioxidant Activity of Solanum xanthocarpum Sehrad. & Wendl.

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Abstract: *The study was done to evaluate the in-vitro antibacterial activity of various extracts was studied and compared with ciprofloxacin as the standard and shows significant activity against E. coli, B. substilis S. aureus, S. pyrogenes, P. aeruginosa, and S. typhi. Anti-fungal activity of the aqueous extract was also studied using miconazole as standard and shows significant activity against A. niger and C. albicans. Anthelmintic activity 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 solvents; petroleum ether (60-80^{o}), chloroform, acetone, ethanol and distilled water. Each extract was selected to study the free radical scavenging activity by DPPH assay 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 44.35 % respectively compared to standard ascorbic acid. Acetone extract showed poor inhibition of DPPH radical compared to standard and other extracts also.*

Keywords: S. xanthocarpum, antibacterial, antifungal, DPPH free radical, phytochemical screening

I. INTRODUCTION

Solanum xanthocarpum Schrad and Wendl is an important medicinal plant belonging 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 (Indian Herbal Pharmacopoeia 1998, The Ayurvedic Pharmacopoeia of India 1989, The Wealth of India (Raw Material) 1972). The roots are used in fever, cough, asthma (Govindan S, et.al. 1999; Bector NP, et.al. 1971; Jain JP, 1980), inflammation and as diuretic & antiemetic. The powdered fruit with honey relieves chronic cough in children. Juice extracted from fruits is used to treat sore throats and the vapours of the burning seeds have been used to relieve toothache (Williamson EM, 1998).8 The extract is also reported for it's antispermatogenic activity in rats (Kanwar U, et.al. 1990; Mali PC, et.al. 1996). It is also reported that fruits and roots showed larvicidal properties against vectors of malaria and dengue (Singh KV, et.al. 2003).

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, perennial herb reaching up to 1.2 m. The leaves are bright green with purplish hairs on both surfaces, ovate or elliptic, sinuate or sub-pinnatified; spines are 1 cm long, sharp, straight and yellow; flowers purple in lateral cymes; berries glabose 1.2 to 2 cm in diameter, green with whitish patches

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when young, yellow and glabrous with green or white veins; seeds glabrous; roots are 3- 5 cm long and 1.5 – 6 mm thick, dull grayish in colour with a soft fibrous fracture stem, flowers and fruits are bitter and carminative (Mali PC, et.al. 1996; Nadkarni KM. 1995; Kirtikar KR, et.al. 1988; Kurup PNV, 1977).

The growing interest in herb and offer economical uses of plant is part of the movement towards "greener" economics and life styles (Karnick CR, 1994). Medicinal plants have attracted attention of not only professionals from various systems of medicine, but also the scientific community belonging to different disciplines. Herbal drugs, being generally harmless in prescribed doses, are becoming popular all over the world and WHO currently encourages, recommends and promotes inclusion of these drugs in national health care programme (Sharma PC, 2002). In recent years, there has been a great interest in herbal remedies for the treatment of number of ailments.

Currently there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Considering the growing interest in assessing the antioxidant capacity of herbal medicine in this research, we studied the antioxidant properties of S. xanthocarpum (Ali SS, 2008).

Medicinal plants are promising source of drugs. S xanthocarpum is a widely available plant in India and very less work has been published so far on antimicrobial, anthelmintic and antioxidant activity. The present study deals with aforesaid activity of the S. xanthocarpum using standard laboratory model.

MATERIAL AND METHODS

Collection and Preparation of Extract

The plant material (whole plant) was collected from Chennai in March 2005 and shade dried. The plant was identified/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).

Shade dried, powdered plant material was extracted in close vessel by maceration process for 72 h at room temperature using petroleum ether (60-80o), chloroform, acetone, ethanol and distilled water (containing chloroform 0.25% v/v as preservative). The extracts were concentrated by distillation under reduced pressure to get solid mass (i.e. completely free from the solvent). 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.

Micro-organisms

Six clinical strains Escherichia coli, Bacillus substilis Staphylococcus aureus, Streptococcus pyrogenes, Pseudomonas aeruginosa, and Salmonella typhi were used for assessing the antibacterial activity with standard ciprofloxacin (10 µg/mL). Two fungal strains Aspergillus niger and Candida albicans were used for antifungal activity determination using Miconazole (10 µg/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 were obtained from Horticulture Department, Panjabrao Deshmukh Krishi Vidhyapith, Nagpur. The cultures were checked for their purity by conventional cultural, morphological and biochemical methods. The bacterial and fungal cultures were maintained and stored in nutrient and Sabouraud's agar medium at 4o C respectively.

Antimicrobial Activity

Antimicrobial activity was determined by the agar cup plate method. Nutrient agar and sabourd dextrose broth were used as medium for bacterial and fungal strain respectively. Control experiments was carried out under the similar condition by using ciprofloxacin (10 µg/mL) and miconazole (10 µg/mL) as standard for antibacterial and antifungal activity respectively. The petridishes with bacteria and fungal cultures were incubated at 37 ± 2o C for 24 hrs. and 27 ±

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2o C for 48 hrs. respectively (Cappucino et.al., 1999). The assessment of antimicrobial activity was based on the measurement of diameter of inhibition zone formed by dissolving the plant material extract in dimethyl sulphoxide (DMSO) and standard drugs also.

Anthelmintic Activity

The anthelmintic activity of S. xanthocarpum was determined by the method of Gunasekaran (2006). The activity was evaluated on adult Indian earthworm - Eudrillus eugeniae. Three groups of approximately equal size worm consisting of six worms in each group were released in 50 mL of desired formulation. Each group was then treated with one of the following- vehicle (1% gum acacia in normal saline), albendazole (25, 50, 100 mg/mL) and ethanolic and aqueous extracts of different concentrations (25, 50, 100 mg/mL).

DPPH radical scavenging activity:

Free radical scavenging activities of all extracts were evaluated for their in vitro free radical scavenging activities by DPPH assay method (Chhajed MR, et al., 2007).

To determine the free radical scavenging activity, a method based on the reduction of a methanolic solution of the coloured DPPH radical was used. To a set of test tubes containing 3 mL of methanol, 50 µL of DPPH reagent (2 mg/mL) was added. The initial absorbance was measured. To these test tubes, methanolic solution of different test solutions (1 mg/mL) were added (10-50 µL). Ascorbic acid (0.5 mg/mL) was also added in the range of 10-50 µL. After 20 minutes, absorbance was recorded at 516 nm. The experiment was performed in triplicate. The percentage reduction in absorbance was calculated from the initial and final absorbance of each solution (Chhajed MR, et al., 2007; Chhajed MR, et al., 2008). Percentage scavenging of DPPH radical was calculated using the formula,

% Scavenging of DPPH = [(Control-Test)/Control] × 100

RESULTS

Phytochemical Investigation

The phytochemical screening of extracts showed the presence of sterols, alkaloids, glycosides, tannins, saponins, phenolic compounds, carbohydrates and proteins in 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.

Antimicrobial activity

The diameter of zone of inhibition is shown in Table 2. Activity index was also calculated (Talesara GL, et.al. 2006) 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.

Anthelmintic activity

Anthelmintic activity of ethanolic and aqueous extracts of S. xanthocarpum against earthworm - Eudrillus eugeniae is shown in Table 3. Ethanolic extract shows significant activity over aqueous extract when compared to standard drug albendazole in same concentration.

Free radical scavenging activity

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%)

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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 shown in figure I.

DISCUSSION:

Antioxidants work to control the levels of free radicals before they do oxidative damage to the body. For example, certain enzymes in the body, such as superoxide dismutase, work with other chemicals to transform free radicals into harmless molecules. Cancer, emphysema, cirrhosis, atherosclerosis and arthritis have all been correlated with oxidative damage (Halliwell B, et.al. 1984). Vitamin C, antioxidant that may prevent cataracts and cancers of the stomach, throat, mouth, and pancreas (D'Mello PM, et.al., 2000). It may also prevent the oxidation of LDL cholesterol, lowering the risk of heart disease.

Geronikaki A, et al., in 2003 reveals that, the carbonyl groups are responsible for free radical scavenging activity. Free Radicals are atoms or group of atoms with an odd (unpair) number of electrons and can be formed when oxygen interacts with certain molecules. Once highly reactive free radical formed they can start a chain reaction like dominoes. Their chief danger comes from the damage; they can do when they react with important cellular components such as DNA, or cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defense system of antioxidant (Nicholls DG, 2000). Antioxidants are able to give free radicals, which becomes a companion to their unpaired electron, thus eliminating the threat of gene alterations leading to cancer (Thomas MJ, 2000; Patil S, et al., 2003).

Free radicals are produced under certain environmental conditions and during normal cellular function in the body. These molecules are missing an electron, giving them an electric charge. To neutralize this charge, free radicals try to steal an electron from, or donate an electron to, a neighboring molecule, creates a new free radical from the neighboring molecule. The newly created free radical, in turn, searches out another molecule and steals or donates an electron, setting off a chain reaction that can damage hundreds of molecules. Antioxidants halt this chain reaction. Some antioxidants are themselves free radicals, donating electrons to stabilize and neutralize the dangerous free radicals. Other antioxidants work against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage (Matill HA, 1947).

This investigation reveals that the Solanum xanthocarpum contains pharmacologically active substance(s) such as alkaloids, glycosides, saponins, flavonoids and phenolic compounds, which are responsible for free radical scavenging properties. The presence of alkaloids, phenolic compounds and flavonoids in the Ethanolic 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. Further studies are in progress in our laboratory to evaluate the in-vivo antioxidant potential of this extract in various animal models and other in-vitro model and phytochemical studies are required to establish the types of compounds responsible for the bioactivity of this medicinal plant and to determine the value of the ethnobotanical approach for the screening of plants as potential source of bioactive substances.

The phytochemical screening of extracts (Harborne JB, et al., 1984) was done 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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Table- 1: Phytochemical Screening of Solanum xanthocarpum

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

Table – 2: Antimicrobial Activity of Solanum xanthocarpum Sehrad. & Wendl.

Extract	Conc	Diameter of Zone of Inhibition in mm[a] (Activity index)[b]							
		Bacteria						Fungus	
		E. coli	*B. substilis*	*S. aureus*	*S. pyrogenes*	*P. aeruginosa*	*S. typhi*	*A. niger*	*C. albicans*
Petroleum ether	100 mg/mL	17 ± .0.76 (0.68)	21 ± 0.90 (0.78)	19 ± 0.95 (0.70)	12 ± 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	100 mg/mL	15 ± 1.44 (0.6)	15 ± 1.32 (0.56)	18 ± 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 ± .093 (0.83)
Acetone	100 mg/mL	16 ± 1.13 (0.64)	18 ± 0.86 (0.67)	21 ± 0.74 (0.78)	15 ± 1.54 (0.63)	12 ± 1.24 (0.46)	20.5 ± 0.88 (0.73)	12 ± 0.95 (0.63)	10 ± 0.96 (0.56)
Ethanol	100 mg/mL	20 ± 1.01 (0.8)	24 ± 0.89 (0.89)	23 ± 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	100 mg/mL	14 ± 0.87 (0.56)	16 ± 0.99 (0.59)	17 ± 0.76 (0.63)	11 ± 0.80 (0.46)	10 ± 1.08 (0.38)	23 ± 1.28 (0.82)	14 ± 1.29 (0.74)	14 ± 0.68 (0.78)
Ciprofloxacin	10 µg/mL	25 ± 0.91	27 ± 1.44	27 ± 0.69	24 ± 1.53	26 ± 1.32	28 ± 1.17	NA	NA
Miconazole	10 µg/mL	NA	NA	NA	NA	NA	NA	19 ± 0.73	18 ± 1.11

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a – an average of three readings, values are expressed in mean ± SEM.
b - (Activity index) = Inhibition zone of the extract / Inhibition zone of the standard
NA: not applied

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

Extract/Drug	Concentration (mg/mL)	Time in minutes for paralysis/death[a]	
		Paralysis	Death
Albendazole	25	20.91 ± 0.50	31.49 ± 0.82
	50	15.66 ± 1.18	28.74 ± 0.62
	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. ± 0.97
	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

Table 4: Free radical scavenging activity of extract of Solanum xanthocarpum

Compd. No.a	% Inhibition of DPPH radicalb	% Free radical scavenging activityb
Standardc	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

a Concentration of test compounds was 50 mg/mL, concentration of standard was 0.5 mg/mL.
b Activity reported at concentration of 50µL of DPPH reagent, values are reported as mean±SEM
c Standard used was ascorbic acid

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