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ANTIBACTERIAL, ANTIFUNGAL AND CYTOTOXIC STUDIES ON LEAF AND SEED EXTRACTS OF *SOLANUM XANTHOCARPUM* SHRAD AND WENDL

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ABSTRACT

Acetone and methanol extracts of leaves and seeds of *Solanum xanthocarpum* were subjected to antibacterial, antifungal and cytotoxic studies. The results indicated significant antibacterial activity of the extracts at 50µg/ml concentration on *Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli* and *Salmonella typhi* but no inhibition of *Pseudomonas aeruginosa and Vibrio cholerae.* The extracts showed marked growth inhibition of *Candida albicans, Aspergillus niger* and *Trichophyton mentagrophytes.* The in vitro study of Human epithelioma of larynx (HEp-2 cell line) cell viability in the presence of leaf extract of S.xanthocarpum showed an IC_{50} value of 625µg/ ml at which concentration the extract was less inhibitory for Green monkey kidney (Vero cell line) cells. The findings of our study will be of great use in developing plant derived antimicrobial and chemotherapeutic agents.

Keywords: antibacterial, antifungal, cytotoxic studies, *Solanum xanthocarpum*, MTT assay

INTRODUCTION

Due to increased emergence of number of antibiotic resistant microorganisms, there is necessity of searching for less toxic antimicrobial agents from plant sources. Antimicrobial agents from natural, especially plant sources may be easily accessible and might be cheaper with minimal side effects.

Cancer is one of the most life-threatening diseases and causes serious health problems in both developed and developing countries. It is a group of diseases characterized by the deregulated proliferation of abnormal cells that invade and disrupt surrounding tissues (Gennari *et al*., 2007). Therefore, investigations for finding new anticancer compounds are imperative and interesting. After taking into consideration the immense side effects of synthetic anticancer drugs, many researchers are making concerted efforts to find new and natural anticancer compounds. The screening of plant extracts has been of great interest to scientists in the search for new drugs for effective treatment of several diseases (Dimayuga and Garcia, 1991).

Drug discovery from medicinal plants has played an important role in the treatment of cancer and indeed, over the last half century most of the plant secondary metabolites and their derivatives have been used toward combating cancer (Newman, 2000; Butler, 2004).

Solanaceae is a large plant family containing two thousand and three hundred species, nearly half of which belong to a single genus, *Solanum*. There are herbs, shrubs and small trees under this genus. This family comprises a number of plants widely known for the presence of variety of natural products of medicinal significance.

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Solanum xanthocarpum Shrad and Wendl (Tamil name: Kandankathri) of the family *Solanaceae* is commonly known as yellow-berried night shade. It is a commonly growing perennial, thorny, herbaceous weed with bright green leaves and zigzag stem, mostly found in arid region.

S. xanthocarpum has profound use in Ayurvedha and folklore medicine. The plant has been extensively used in traditional medicine in India to cure liver disorders, chronic skin ailments (psoriasis and ringworm), inflammatory conditions, dysmenorrhea, fever, diarrhea, eye diseases, and hydrophobia (Kritikar and Mohan Basu, 1935). Crude plant extract is beneficial in bronchial asthma and non-specific cough, influenza, difficult urination, bladder stones, rheumatism etc.

MATERIALS AND METHODS

Test Organisms

Staphylococcus aureus, Escherichia coli, Aeromonas hydrophila, Salmonella typhi and *Vibrio cholerae* were used to test anti bacterial activity and *Candida albicans, Trichophyton mentagrophytes, and Aspergillus flavus* were used to assess antifungal activity. All the stock microbial cultures were obtained from Microbial Type Cell Culture (IMTECH, India).

Plant material

S. xanthocarpum plants were obtained from Anna medicinal farm, Chennai. The seeds and leaves were washed with distilled water, dried in shade, finely powdered & stored in air tight containers.

Preparation of plant extract

25 g of air-dried leaf and seed powders of *S. xanthocarpum was* immersed in 100 ml of acetone and methanol in separate 250 ml Erlenmeyer flasks. The flasks were incubated at room temperature for 48 h at 150 r.p.m in an orbital shaker. The suspension was filtered and concentrated to dryness at 40^{o}C in hot air oven. The extract was dissolved in Dimethyl sulphoxide (DMSO) to a concentration of 100mg/ml. The range of working concentrations prepared from stock solution was 2.5, 5, 10, 15 and 20 mg/ml.

Assay of antibacterial activity

Preparation of active bacterial suspension

Stock bacterial cultures were maintained at 4°C on nutrient agar slants. Active cultures for experiments was prepared by transferring a loopful of stock culture to 10 ml of nutrient broth and incubating aerobically at 37° C for 24 h for bacterial proliferation.

Determination of antibacterial activity of plant extracts

The effect of the plant extracts against different bacteria was determined by Kirby-Bauer disc diffusion method. With sterile cotton swabs the test bacterial suspensions, with a turbidity matching to 0.5 McFarland standard (as read in a spectrophotometer at 530_{nm}), with bacterial concentration equivalent to 1 to 5 x 10^{6}/ ml, were inoculated on Mueller-Hinton agar plates. Whatman No 3 filter paper discs (5 mm diameter) were impregnated with 20 µl of different concentration of each plant extract, placed on the surface of the test plates and incubated at 37ºC for 24 h.

Assay of antifungal activity

Preparation of working fungal cultures

Antifungal activity of the extracts was determined by antifungal susceptibility test. Potato dextrose broth tubes were inoculated with the stock fungal cultures. Then the tubes were kept in an orbital shaker (150 r.p.m) for up to 4 days at 30°C.

Preparation of medium

3.9gm of potato dextrose agar powder was weighed and dissolved in 100 ml of distilled water. The medium was autoclaved at 121°C for 15 min. After sterilization 1000µl of 4 different solvent extracts were added to four different Erlenmeyer flasks except for the control flask. The mixture was poured in to sterile Petri plates, and the medium left in the laminar air flow chamber for 30-60 min. After solidification 20µl of the fungal cultures were inoculated to the Petri plates and incubated at 30°C. Amphotericin B at a concentration of 16µg/ml (in DMSO) was used as the control. The growth inhibition was observed for 2-4 days.

Anticancer drug screening

The antitumor assay was performed on Human laryngeal epithelioma cells (HEp-2 cell line) obtained from King Institute of Preventive Medicine, Chennai, India, with non-toxic dose of the plant extract and its dilutions.

Cytotoxicity assay by cell viability test

The assay was carried out using (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial enzyme dehydrogenase of viable cells, yielding a measurable purple product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity (Mosmann, 1983; Butler, 2004).

Preparation of working herbal extracts

0.5ml of stock (100 mg/ ml) herbal extract was dissolved in 4.5 ml of DMSO for a concentration of 10 mg/ ml. The fresh working suspension filtered through 0.45 µm membrane filter prior to the assay.

Using the 10mg/ ml concentration herbal extract nine serial doubling dilutions of the extract of 500µl each was prepared in DMSO to get the concentration of the extract as indicated in Table-3 and the diluted extracts transferred to 10 wells of a 12 well culture plate. 500 µl of 48h culture of HEp-2 cells at a concentration of 10^5 cells/ ml was added to each well. Two control wells received only cell suspension without plant extract. The plate incubated in a humidified CO_2 incubator at 37° C for 4 - 6 h. The plate was microscopically examined for confluent monolayer of cells, turbidity and toxicity.

MTT assay (Taylor *et al*. 1996; Quintero *et al*. 1999)

After incubation, the medium from the wells aspirated carefully and discarded. Each well washed with Eagle's Minimum Essential Medium (EMEM) without Fetal Calf Serum (FCS). 200µl of MTT solution (5mg MTT/ ml of PBS, pH 7.2) added to each well. The plate incubated for 6-7 h at 37°C in a CO_2 incubator with 5% CO_2. After incubation 1ml of DMSO added to each well and mixed with pipette and left for 45s at room temperature. Purple formazan formed in the wells. Cell control and solvent control were included in each assay to compare the full cell viability in cytotoxicity and antitumor activity assessments. The suspension transferred to a spectrophotometer cuvette and the optical density (O.D) measured at 540_{nm} using DMSO as blank. The % cell viability was calculated with the following formula:

Cell viability % =

Mean OD_{540} of wells receiving each plant extract dilution/ Mean OD_{540} of control wells x 100

The assay was performed in triplicates for each of the extracts. The mean of the cell viability values was compared to the control to determine the effect of the extract on cells. The minimum concentration of plant extract that was non-toxic to Vero cells but toxic to HEp2 cells was recorded as the effective drug concentration.

RESULTS

Antibacterial activity

The antibacterial activity of the different solvent extracts of *S. xanthocarpum* showed significant variations as shown in Table.1.Among the two extracts tested, methanol extract had greater antibacterial potential, followed by Acetone extract. The largest zones of inhibition were observed for methanol leaf extract against *S. typhi* (20 mm) and S. *aureus* (19 mm). No activity was observed with acetone and methanol seed extracts against *P.aeruginosa* and *V.cholerae.* The methanol extracts of different parts of *S. xanthocarpum* showed antimicrobial activity against all the test microorganisms with the inhibition zone ranging from 14 to 20 mm respectively. The activities are comparable with the reference drug Ampicillin (10µg/ml).

Table 1- In vitro antimicrobial activity of extracts of *Solanum xanthocarpum.* (Zone of inhibition in mm)

S. No	Plant extracts	Micro organism	50µg	25 µg	10 µg	Ampicillin 10 µg/ml (Control)
1.	**Acetone leaf extract**	*A.hydrophila*	15	14	10	15
		E. coli	16	14	12	16
		S. aureus	19	15	12	24
		S. typhi	16	14	11	17
		P.aeruginosa	10	-	-	10
		V.cholerae	12	09	-	14
2.	**Acetone seed extract**	*A.hydrophila*	15	10	-	15
		E. coli	15	14	11	16
		S. aureus	15	10	08	24
		S. typhi	15	10	-	17
		P.aeruginosa	-	-	-	10
		V.cholerae	-	-	-	14
3.	**Methanol leaf extract**	*A.hydrophila*	16	14	10	15
		E. coli	15	13	12	16
		S. aureus	15	12	09	24
		S. typhi	18	16	13	17
		P.aeruginosa	-	-	-	10
		V.cholarae	10	-	-	14
4.	**Methanol seed extract**	*A.hydrophila*	17	14	08	15
		E. coli	14	12	07	16
		S. aureus	19	16	10	24
		S. typhi	20	17	12	17
		P.aeruginosa	-	-	-	10
		V.cholerae	-	-	-	14

Antifungal activity

The antifungal activity of the solvent extracts of *S. xanthocarpum* also varied significantly among the test organisms as shown in Table 2. All the extracts inhibited the growth of *Candida albicans* and the growth of *Aspergillus flavus but* as the incubation time was prolonged, the extract showed higher inhibitory activity against *Trichophyton mentagrophytes.* Acetone extracts of leaf and seed showed better inhibition of the fungal pathogens compared to methanol extracts.

Cytotoxic activity

The OD readings are shown in Tables 3 and 4. The MTT assay showed an anti proliferative activity (IC_{50}) of HEp-2 cells at 625µg/ ml of leaf extract of *S.xanthocarpum*. The MTT assay of acetone leaf extract of *S. xanthocarpum* treated wells showed 625 µg/ ml as the effective drug concentration. At this concentration the extract was non-toxic to Vero cells and also caused more than 50% cytotoxicity to HEp-2 cells.

Table 2 - Antifungal activity of the extracts of *Solanum xanthocarpum.* (Zone of inhibition in mm)

S.No	Microorganism	Duration (h)	Acetone	Methanol	Amphotericin B (16µg/ml) (Control)
	Leaf Extract				
1.	*C.albicans*	48	8.5	6.5	10
2.	*A.flavus*	48	15	11	20
3.	*T. mentagrophytes*	48	11	9.5	14
	Seed Extract				
4.	*C.albicans*	48	9	7	10
5.	*A.flavus*	48	16	12.5	20
6.	*T. mentagrophytes*	48	11	9	14

Table 3 – HEp-2 cell viability with different concentrations of acetone extract of leaves of Solanum xanthocarpum.

S. No	Concentration (mg /ml)	Dilutions	Absorbance	Cell viability %(HEP-2 cells)
1	10	Neat	0.06	11.76
2	5	1:1	0.14	27.45
3	2.5	1:2	0.17	33.33
4	1.25	1:4	0.22	43.13
5	0.625	1:8	0.29	56.86
6	0.3125	1:16	0.31	60.78
7	0.156	1:32	0.38	74.50
8	0.078	1:64	0.44	86.27
9	0.039	1:128	0.48	94.11
10	Cell control	-	0.51	100

Table 4 – Vero cell viability with different concentrations of acetone extract of leaves of *Solanum xanthocarpum.*

S. No	Concentration (mg/ml)	Dilutions	Absorbance	Cell viability % (Vero cells)
1	10	Neat	0.15	25.42
2	5	1:1	0.25	42.37
3	2.5	1:2	0.29	49.15
4	1.25	1:4	0.35	59.32
5	0.625	1:8	0.43	72.88
6	0.3125	1:16	0.46	77.96
7	0.156	1:32	0.49	83.05
8	0.078	1:64	0.54	91.52
9	0.039	1:128	0.57	96.61
10	Cell control	-	0.59	100

DISCUSSION

Many plants have antimicrobial principles such as tannins, catechins, essential oils, alkaloids (20%) steroids & triterpenoids (25%) saponins (45%) and polyphenolic acids (Arthur, 1954; Deininger, 1984). Phytochemical analysis of *S.xanthocarpum* revealed the presence of solasonine, solasomargine, sapogenin and solasodine that are responsible for the medicinal effect of this plant (Linday, 1962; Oudhia and Kadu Pani, 2007; *Diallo, 1999; Rojas, 2006; Erdogrul, 2002*). Lipophilic flavonoids disrupt microbial cell membranes. Some phytochemicals form a complex with extracellular, soluble microbial proteins which bind to the microbial cell wall resulting in the dissolution of the cell wall (Tsuchiya *et al*., 1996).

Plant based compounds have been playing an important role in the development of several clinically useful anti-cancer agents such as vinblastine, vincristine, camptothecin derivatives, topotecan, irinotecan, etoposide derived from epipodophyllotoxin and taxol (Shoeb, 2006). Our study indicates the scope of developing antimicrobial and anticancer drugs from *S.xanthocarpum*. This requires further in vivo animal experiments and human trial studies before such phytocompounds are approved for human therapeutic use.

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