Biological Activities of Various Crude Fractions of *Solanum xanthocarpum*'s Fruits

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Summary: Crude methanolic, and non-alkaloidal chloroform, hexane and methanol extracts of the fruits of *Solanum xanthocarpum*, were tested for their *in vitro* antifungal, antibacterial, leishminicidal, insecticidal, cytotoxic and antiglycation activities. Crude non-alkaloid methanol extract showed highest activity against *T. longifuses* and *M. canis*, non-alkaloidal chloroform extract was found active against *A. flavus* while all other fractions showed weak activities or found inactive. Crude methanolic extract was found moderately active against *S. Typhi* while, all the other fractions were found inactive against all the bacterial cultures. Crude methanolic extract showed moderate cytotoxicity while all the other fractions were non cytotoxic. No insecticidal activity was found in any fraction, while, crude methanolic fraction exhibited moderate anti-glycation and leishminicidal activities.

Introduction

Antibiotic resistance has become a global issue and consequently the clinical efficacy of many existing antibiotics is being threatened by emergence of multi drug-resistant pathogens [1, 2]. Many infectious diseases are treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drugs because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial agents with diverse chemical structures and novel mechanisms of action. Therefore, researchers are increasingly turning their attention to herbal medicine, looking for discovery of new leads to develop better drugs against microbial infections [3].

Solanum xanthocarpum belongs to the family Solanaceae and commonly known as the Indian night shade or yellow berried night shade and locally known in pushto as Maraghony. Various parts of the Solanum xanthocarpum find its applications in curing a variety of ailments, which include fruit juice for sore throats and rheumatism, decoction for gonorrhea, paste of leaves is applied to relieve pains, seeds are expectorant in cough and asthma and roots are used for diuretic, catarrhal fever, and chest pain [4].

Sterols, alkaloids, glycosides have been earlier isolated from the plant. Solasodine, an antiandrogenic alkaloid, diosgenin and β- sitosterol are also reported from the fruits of *Solanum xanthocarpum* [5, 6]. Seeds contain 5-7 % oil [7]. The plant has also exhibited pest repellent properties and has been exploited as contact poison and molluscicide [8]. This plant is reported for significant antifungal activity against pathogenic *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*, moreover, it shows ideal larvicidal activities against *Anopheles stephensi* and *Culex quinquefasciatus* [9, 10]. A decoction of the fruits of this plant is used by tribal and rural people of Orissa, India for the treatment of diabetes [11-13].

Result and Discussion

Literature describes the methanol extracts of *Solanum xanthocarpum* and *Datura metel* inhibited the growth of *A. fumigatus*, *A. flavus* and *A. niger*

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having MIC's values 1.25-2.50 mg ml^{-1} by both micro broth dilution and percent spore germination assays [10]. More recently Salar *et al.*, have reported antibacterial activity of all part of the plant against selected bacteria [14]. This prompted us to investigate antifungal activity of various crude fractions of *Solanum xanthocarpum*. The crude non-alkaloidal hexane extract of *Solanum xanthocarpum* exhibited good inhibitory activity against *Trichophyton longifusus and Microsporum canis* (80 % and 60 %, respectively) while, crude alkaloid-free chloroform extract was found to be moderately active against *Trichophyton longifusus*, *Microsporum canis* and *Aspergillus flavus showing inhibition rate* 20 %, 40 % and 40 % respectively. Moderate inhibitory activity (20 % and 40 %) was observed for the crude alkaloid free methanol extract against *Trichophyton longifusus* and *Microsporum canis* respectively (Table-1).

Table-1: Antifungal activities of crude extract of *Solanum xanthocarpum* (% Inhibition).

Name of fungus	Fr.1	Fr.2	Fr.3	Fr.4	Standard drug [a, b]
Trichophyton longifusus	20	80	20	10	70[a]
Candida albican	0	0	0	0	110.8[a]
Aspergillus flavus	40	0	0	0	20[b]
Microsporum canis	40	60	40	8	98.4[a]
Fusarium solani	0	0	0	0	73.25[a]
Candida glabrata	11	0	8	0	110.8[a]

Abbreviations: Fr.1 (crude non-alkaloidal chloroform extract), Fr.2 (crude non-alkaloidal hexane), Fr.3 (crude non-alkaloidal methanol), Fr.4 (crude methanol extract); standard Drug; a = Miconazole, b=Amphotericin-B

The literature describing phyto-chemical and antimicrobial studies of Solanacae revealed that non-alkaloid constituents of some Solanaceous fruits including *Solanum xanthocarpum* exhibited low bactericidal activities against *Bacillus subtilis*, *Staphylococcus aureu and Eschericha coli* [15]. We were therefore interested to test in vitro antibacterial activities of various alkaloid free and alkaloidal crude extracts, which would be highly helpful in bioassay guided isolation of pure compounds in search of better antibacterial agents. Antibacterial activity was done at 2 mg/mL. All the extracts were found inactive against all the bacterial strains, while the crude methanol extract was found weakly active against *Salmonella typhi* (Table-2). Crude extracts were also screened for cytotoxicity. Almost all the fractions were found non-toxic with the exception of crude methanol extract Fr.4 (Table-5), which showed weak cytotoxicity (LD_{50} (µg/ml) = 350.8944 µg/ml).

Crude methanol extract showed moderate anti-glycation activity at 500 µg concentration

Table-2: Antibacterial activities at 3mg/ml of crude extract of *Solanum xanthocarpum* (% Inhibition).

Name of Bacteria	Fr.1	Fr.2	Fr.3	Fr.4	Imepinum
E. Coli	--	02	--	--	25
B. subtilis	--	--	03	--	26
S. Flexenari	--	--	--	06	24
S. aureu	04	--	--	--	17
p. aeruginosa	--	04	--	--	17
S. Typhi	03	--	--	10	21

Abbreviations: Fr.1 (crude non-alkaloidal chloroform extract), Fr.2 (crude non-alkaloidal hexane), Fr.3 (crude non-alkaloidal methanol), Fr.4 (crude methanol extract); Imepinum = standard drug

(Table-3). Leishmania has been shown to be a major public health problem, mostly in Africa, Asia and Latin America according to world health organization report (WHO) and is of serious concern. Therefore, crude metahnolic extract was also screened for leishmanicidal activity and was found moderately active against leishmanial promastigotes. The extracts were also tested for their insecticidal activities but all the extracts were found non insecticidal.

Table-3: Antiglycation activity of crude methanol extract of *Solanum xanthocarpum* (% Inhibition).

Concentration (µg)	Inhibition (%)	IC_{50} ± SEM (mM)
500	71.6	57.1 (± 40.1)

Table-4: leishminicidal activity of crude methanol extract of *Solanum xanthocarpum*.

leishminicidal activity	IC_{50} (µg mL^{-1}) ± S.D
500 µg	57.1 (± 40.1)
Amphotericine B. (Std. Drug)	0.50 (± 02.1)

Table-5: Cytotoxicity *Solanum xanthocarpum* (% Inhibition).

Fraction	dose (µg/ml)	No. of shrimps	Shrimps survived	LD_{50}(µg/ml)
Fr.1	1000	30	27	--
	100	30	27	
	10	30	29	
Fr. 2	1000	30	29	---
	100	30	30	
	10	30	30	
Fr.3	1000	30	26	–
	100	30	28	
	10	30	28	
Fr.4	1000	30	7	350.8944
	100	30	25	
	10	30	29	
Etopside				7.4625

Abbreviations: Fr.1 (crude non-alkaloidal chloroform extract), Fr.2 (crude non-alkaloidal hexane), Fr.3 (crude non-alkaloidal methanol), Fr.4 (crude methanol extract); Etopside = standard drug

Experimental

Plant Collection

Solanum xanthocarpum collected in mature stage from its natural habitat in Peshawar in the

month of July and August. Plant was identified in Medicinal Botanic Center, Pakistan Council of Scientific and Industrial Research, Peshawar and a voucher specimen (7938 PES) was deposited at the herbarium of the same department.

Removal of Alkaloids

Powdered dried fruits of *Solanum xanthocarpum* (4.5kg) was soaked in aqueous H_2SO_4 (5 %, 3 L) for three days to remove the alkaloids adopting the standard procedure [14]. Suspended material was stirred occasionally and collected on Buchner funnel, washed with water (4x700 ml) followed by 5 % w/v Na_2CO_3 solution (2x700 ml). The alkaloid free residue, dried in open air was further extracted with hexane, chloroform and methanol.

Extraction

The powdered plant material (4.5kg) and alkaloids free powdered residue (4kg) were separately extracted with hexane, chloroform and methanol respectively. The solvent after each extraction was evaporated at low temperature under reduced pressure in rotary vacuum evaporator to obtain crude extracts.

Bioassay

Antifungal Bioassay (in vitro)

Crude extracts were screened for their antifungal activity using agar tube dilution method [16] against *Trichophyton longifusus*(clinical isolate), *Candida albican* (ATCC 2091), *Aspergillus flavus* (ATCC 32611), *Microsporum canis*(ATCC 11622), *Fusarium solani*(ATCC 11712), *Candida glabrata* (ATCC 90030). The test sample was prepared by dissolving 24mg of crude extract in 1.0 ml sterile DMSO, serving as stock solution. Sabouraud dextrose agar (SDA, 4ml) was dispensed into screw cap tubes and autoclaved at 121°C for 15 minutes and then cooled to 50°C. The non-solidified SDA was loaded with 66.6 µl of compound pipette out from the stock solution. This gave the final concentration of 400 µg ml^{-1} of the crude extract in the media. They were allowed to solidify at room temperature. Each tube was inoculated with 4mm diameter piece of inoculum removed from a seven-day-old culture of fungus. For non-mycelial growth, an agar surface streak was employed. Other media supplemented with DMSO and reference antifungal drugs was used as negative and positive control respectively. The tubes were incubated at 27-29 °C for 7-10days. Growth in the media was determined by measuring linear growth (mm) and the percent inhibition was calculated with reference to the negative control.

Antibacterial Bioassay (in vitro)

The crude extracts were screened for antibacterial activity against the selected bacterial strains including; *Escherichia coli* (ATCC 25922), *Bacillus subtilus* (ATCC 6633), *Shigella flexenari* (clinically isolated), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *and Salmonella typhi* (ATCC 19430) using literature protocol [17]. In the agar well diffusion method, wells were dugged in the media with the help of a sterile metallic borer with centers at least 24 mm part. 2-8 hours old bacterial inoculums containing approximately 10^4-10^6 colony forming units (CFU)/mL were spread on the surface of nutrient agar with the help of a sterile cotton swab. Cotton swab was rotated firmly against the upper inside well of the tube to express excess fluid, entire agar surface of the plate was streaked with the swab three times turning the plate 60° between each streaking. Recommended concentration of the test sample (2mg/mL of DMSO) was then added in their respective wells. Other wells supplemented with DMSO and reference antibacterial drug (Imipenum, 100 µg ml^{-1}) serving as negative and positive controls, respectively. The plates were incubated immediately at 37 °C for 14-19 h. Potency was determined by measuring the diameter of zones showing complete inhibition (mm).

Brine-Shrimp Lethality Bioassay

Artemia salina (brine shrimp eggs) was used to determine the cytotoxic activity of the various samples [18, 19]. Three different concentrations (10, 100 and 1000 µg/ml) of test samples were prepared by dissolving in DMSO as the solvent. Seawater was prepared by dissolving commercially available sea salt (3.8g) into tap water (1:1). Brine shrimps hatched in seawater media at 27oC for 48 h. Ten shrimps; seawater (5 ml) and different amounts of each sample were put in a vial. Two other vials were supplemented with solvent and reference cytotoxic drug serving as negative and positive controls

respectively. Etoposide (LD_{50} = 7.465 μg ml^{-1}) was used as the standard reference cytotoxic drug. All vials were incubated at 25-27 °C for 24 h. and the survived brine shrimps were counted. The data was analyzed with Finney computer program to determine LD_{50} values with 95% confidence intervals.

Insecticidal Assay

The crude extracts were examined for their insecticidal activity against *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Callosbruchus analis* and *Trogoderma granarium* [20, 21]. Test samples (1000, 500, 100, 50 and 10ppm) were dissolved in methylen chloride and these solutions were then coated on the inner surface of 20 ml glass vials (two to five replicates for each concentration). Each glass vial was rotated by hand until the test solution was distributed on the vial inner wall and floor, and the solvent had mostly evaporated. Then each vial was placed in a fume hood for 10 minutes to ensure complete removal of the carrier solvent. When the solvent was completely evaporated, five test insects (or larvae) were placed carefully in each vial with sufficient food (*i.e.* the natural diet for that particular insect, such as leaves, grain, etc., or artificial diet which is different for different insects). The survival of the insects was assessed after 24-48 h. Controls consist of test insects (or larvae) in vials, treated only with the carrier solvent. The concentration of each sample was taken as 1019.10μg cm^{-2}. The standard drug used was permethrin and its concentration 235.9μg cm^{-2} Survival of such controls should average over 95 %. Data was analyzed with a Finney computer program (Probit analysis) to determine LC_{50} values and 95 % confidence intervals.

Antileishmanial Assay (in vitro)

Promastigotes are the extracellular flagellated form of the parasite and they can be used for *in vitro* determination of leishmanicidic activity of natural compounds. [22, 23]. Leishmanial promastigotes were cultured in sterile 25 cm^2 tissue culture flask in tissue culture medium M-199 supplemented with 25 mM HEPES and 10% HIFBS (heat inactivated foetal bovine serum) at 25°C. Parasites were centrifuged at 3000 rpm, diluted in minimum volume of PBS and were counted with the help of improved Neubauer chamber under a microscope. Parasites were diluted with the fresh medium to a final concentration of 2.0 x 10^6 parasites ml^{-1}. One mg of compound was dissolved in 50μl of absolute MeOH or DMSO (Dimethyl sulfoxide) and the volume was made up to 1.0 ml with the culture medium. In a 96 well microtiter plate, 90μl of the parasite culture (2.0x 10^6 parasites ml^{-1}) was placed and 10μl containing various concentrations of the experimental compound was added in the culture. Ten ul of PBS (phosphate buffered saline, pH 7.2 containing 0.5 % DMSO) was added as negative control while amphotericin B, and pentamidine (to a final concentration of 1.0 mg ml^{-1}) were added separately as positive control. The plates were incubated at 25°C in the dark for 3-5 days during which control organism multiplied 3-6 times. The culture was examined microscopically on an improved Neubauer chamber and ED_{50} value of compounds possessing antileishmanial activity was calculated.

Conclusion

A detailed study on the biological usefulness including; anti-fungal, antibacterial, leishminicidal, insecticidal and cytoxiocity and antiglycation activities of *Solanum xanthocarpum* has been carried out. Crude non-alkaloidal chloroform extract, crude non-alkaloidal hexane, crude non-alkaloidal methanol exhibited good to moderate antifungal activity, no significant antibacterial and insecticidal activities were found. Only crude methanol extract showed weak cytotoxic, moderate leishminicidal and good antiglycation activity. In conclusion methanol and chloroform fractions could be exploited for the potential bioactive antifungal and leishminicidal agents.

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