

Anti-microbial, Anti-oxidant and Anthelmintic Activity of Crude Extract of *Solanum xanthocarpum*

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ABSTRACT

The aim of the present study was to evaluate the antimicrobial, anti-oxidant and anthelmintic potential of extracts of the herb *Solanum xanthocarpum*. The antimicrobial activity was tested against selected bacterial and fungal species by agar well diffusion method and the antioxidant activity was assessed using FRAP and reducing power scavenging assays. While that of anthelmintic activity was performed against the earthworms. The FRAP assay evaluates the ability of a substance to reduce Fe^{3+} to Fe^{2+} . Preliminary phytochemical screening revealed the presence of saponins; phytosterols and carbohydrates in the plant.

Keywords: Agar well diffusion method, anthelmintic activity, antimicrobial activity, anti-oxidant activity, FRAP, *Solanum xanthocarpum*

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INTRODUCTION

Dependence on herbs as medicine in the treatment of diseases is still much practiced by a large proportion of the rural population because of its availability and affordability. Due to increased awareness of the importance of traditional medicine in human and animal health care, research into the efficacy of some of the herbs used in the treatment of some illness would be worthwhile. In every society, whether technologically primitive or advanced, there exist some sort of curative recipes for the health maladies.^[1]

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc.^[2]

Solanum xanthocarpum Schrad. & Wendl. (Solanaceae) commonly known as Yellow Berried Nightshade (syn:

kantakari), is a prickly diffuse bright green perennial herb, woody at the base, 2–3m height found throughout India, mostly in dry places as a weed on roadsides and waste lands.^[3] The fruits are glabrous, globular berries, green and white strips when young but yellow when mature.^[4] The fruits are known for several medicinal uses like anthelmintic, antipyretic, laxative, anti-inflammatory, antiasthmatic and aphrodisiac activities.^[5] The stem, flowers and fruits are prescribed for relief in burning sensation in the feet accompanied by vesicular eruptions.^[6] The hot aqueous extract of dried fruits is used for treating cough, fever and heart diseases.^[7] The fruit paste is applied externally to the affected area for treating pimples and swellings.^[8] The *kondh* tribes of Dhenkanal district of Orissa, India uses the hot aqueous extract of the matured fruits as a traditional medicine for the treatment of diabetes mellitus. The fruits are reported to contain several steroidal alkaloids like solanacarpine,^[9] solanacarpidine, solancarpine, solasonine^[7] and solamargine.^[10] Other constituents like caffeic acid^[10] coumarins like aesculetin and aesculin,^[11] steroids carpesterol, diosgenin, campesterol, daucosterol and triterpenes like cycloartanol and cycloartenol were reported from the fruits.^[12,13] The antispasmodic, antitumor, cardiotoxic, hypotensive, antianaphylactic and cytotoxic activities were also reported.^[14-16] In the present communication we report the hypoglycaemic

activity of the aqueous extract of the fruits of *Solanum xanthocarpum*.

Free radicals are produced in normal and/or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage.^[17] Almost all organisms are well protected against free radical damage by oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or chemical compounds such as α -tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione.^[18] When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur, resulting in diseases and accelerated ageing. However, antioxidant supplements or antioxidant containing foods may be used to help the human body to reduce oxidative damage.^[17,19-21]

Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals (O_2^-), hydroxyl radicals (OH^\cdot), singlet oxygen (1O_2) and non-free radical species such as hydrogen peroxide (H_2O_2) are various forms of activated oxygen and often generated by oxidation product of biological reactions or exogenous factors.^[21-23] ROS are continuously produced during normal physiologic events, and removed by antioxidant defence mechanisms.^[24] There is a balance between generation of ROS and antioxidant system in organisms. In pathological condition, ROS are over produced and result in lipid peroxidation and oxidative stress. The imbalance between ROS and antioxidant defence mechanisms leads to oxidative modification in cellular membrane or intracellular molecules.^[25] Various endogenous antioxidant defence mechanisms play an important role in the elimination of ROS and lipid peroxides, and therefore, protect the cells against toxic effects of ROS and lipid peroxides.^[24-26]

Vegetables and fruits are considered to be good sources of functional ingredients. Many studies have shown that antioxidants, present in plants at high levels, are the compounds responsible for these functionalities.^[27] Antioxidants or molecules with radical scavenging capacity are thought to exert a potential protective effect against free radical damage. These biomolecules contribute to prevention of coronary and vascular diseases and of

tumor formation by inhibiting oxidative reactions.^[28] This oxidative damage is the result of free radical action on, for instance, lipids or DNA.^[29]

The antioxidant activity of some of these molecules is based on their ability to donate a hydrogen atom to free radicals. Because these compounds are able to scavenge free radicals, they are believed to have potential in the prevention of cancer, atherosclerosis, and diabetes.^[30] Nowadays there is considerable evidence that the antioxidants contained in fruits, vegetables and beverages play an important role in the maintenance of health and in prevention of disease.

Helminthic infections of the gastrointestinal tract of human beings and animals have been recognized to have adverse effects on health standards with a consequent lowering of resistance to other diseases. In search of compounds with anthelmintic activity, a number of substances were screened using different species of worms, for example, earthworms, *Ascaris*, *Nippostrongylus*, and *Heterakis*. Of all these species, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* because they resemble intestinal "worms" in their reaction to anthelmintics and are easily available. It has been demonstrated that all anthelmintics are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as an anthelmintic.^[31]

MATERIAL AND METHOD

Plant Material

The fresh whole plants of *Solanum xanthocarpum* were collected from the rural area. The plant was identified and authenticated by Dr. Ms. Sattarupa Rao, Professor and Head, Department of Crop and Herbal Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.). Specimen Voucher no. HD/CHPY/1138.

Preparation of Extract

The plant was shade dried at room temperature and the dried whole plant was ground into coarse powder. The powder was sieved to have a uniform size. The aqueous, ethanolic and hydroethanolic extracts were obtained by cold maceration. The extraction procedure was repeated thrice in order to have optimum extraction. The extracts were filtered using a muslin cloth and concentrated at $40 \pm 5^\circ C$; dried extracts were refrigerated at $4^\circ C$ until use.

Phytochemical Analysis

The phytochemical analysis of the plant was carried out by the standard methods^[32,33] (Table 1).

Determination of Anti-oxidant Property Chemicals

Safranine, potassium ferricyanide, trichloroacetic acid, ferric chloride, ascorbic acid were purchased from CDH New Delhi. The other chemicals and reagents used were of analytical grade.

Ferric Reducing Antioxidant Potential (FRAP) Assay

Ferric reducing ability was evaluated using different concentrations of crude extract. The FRAP reagent contained 10mM of TPTZ solution in 40mM HCl, 20mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and acetate buffer (300mM, pH 3.6) (1:1:10, v/v/v). A 100 μL 50% aqueous methanol of the test compounds was added to 3mL of the FRAP reagent, and the absorbance was measured at 593nm after incubation at room temperature for 6min, using the FRAP reagent as a blank. Different dilutions of each of the test compounds were assayed and the results were obtained by interpolating the absorbance on a calibration curve. The FRAP value was defined as the milliequivalents of Trolox having the antioxidant power equivalent to a 1.0mM solution of the substance under study. Two independent experiments in triplicate were performed for each of the assayed compounds.^[34]

Scavenging Activity against Hydroxyl Radicals

Different concentrations of extract were prepared and 1 ml of each aliquot were mixed with 1ml of 10 mMol/L phosphate buffer solution (pH 7.4), 1 ml of 40 $\mu\text{g}/\text{ml}$ safranine T solution, 1 ml of 3ml/100ml of H_2O_2 and 1.0ml of 0.15 mol/L EDTA, FeNa. This reaction mixture was incubated at 37°C for 30 min. same procedure is repeated with control.

Determination of Anti-microbial Property Microorganisms

Bacterial and fungal cultures used in the present studies were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. The bacterial strains were *Escherichia coli* MTCC 2960, *Pseudomonas aeruginosa* MTCC 4676, *Staphylococcus aureus* MTCC 3160, *Klebsiella oxytoca* MTCC 3030, *Bacillus subtilis* MTCC 1790, *Candida albicans* MTCC 183.

Preparation of Inoculums

All the microorganisms mentioned above were incubated at $37 \pm 0.1^\circ\text{C}$, for 24 h in Nutrient broth, *C. albicans* in YEPD broth at $28 \pm 0.1^\circ\text{C}$ for 48 h.

Determination of Antimicrobial Activity

Nutrient Agar and YEPD Agar (20 ml) were poured into each sterilized Petri dish (10 X 100 mm diameter) after injecting cultures (100 μl) of bacteria and yeast and distributing medium in Petri dishes homogeneously. For the investigation of the antibacterial and anticandidal activity, the dried herb extracts were dissolved in dimethylsulfoxide to a final concentration of 20% and sterilized by filtration through a 0.22 μm membrane filter.^[35,36] Each sample (100 μl) was filled into the wells of agar plates directly. Plates injected with the fungal cultures were incubated at 28 °C for 48 h, and the bacteria were incubated at 37°C for 24 h. At the end of the incubation period, inhibition zones formed on the medium were measured in mm. Studies were performed in triplicate and the inhibition zones were compared with those of reference discs. Amphotericin B (10 μg) and tetracycline (30 μg) were taken as reference.

Determination of Anthelmintic Activity Standard

Piperazine citrate (10mg/ml) was used as reference standard

Worm Collection and Authentication

The earthworm *Eisonia fatida* (African type) were collected and authenticated from Madhya Pradesh Pashu Chikitsa Vishwavidyalaya, Jabalpur (M.P.), India.

Anthelmintic Activity

The anthelmintic assay was carried using different concentrations of crude aqueous, ethanolic and hydroethanolic extracts (25, 50, and 100mg/ml in distilled water) were prepared, and three worms of nearly same type per concentration were placed in it. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water(50°C).^[37]

Piperazine citrate (10mg/ml) was used as reference standard and distilled water served as the control.

RESULT AND DISCUSSION

Anti-microbial activity

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay.^[38] Many reports are available on the antiviral,

antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants.^[39-45] The antimicrobial activity of extracts of *S. xanthocarpum* was tested under *in vitro* conditions by agar well diffusion method against three bacterial and one fungal pathogen. The process was performed in triplicates. The zone of inhibition of microbial growth with Hydroethanolic extracts is given in Table 2. The aqueous, hydroethanolic and ethanolic extract of *S. xanthocarpum* (50 µg/ml) inhibited the growth of Gram negative bacteria *S. aureus* (10, 15 and 13mm respectively) and *E. coli* (6, 8 and 7mm respectively). Solvent extracts *S. xanthocarpum* (50 µg/ml) exhibited mild to moderate inhibition over the growth of tested bacterial pathogens. Ethanolic extract was found to be having more potent anti-microbial activity than those of aqueous and hydro ethanolic extract (Table 2).

Anti-oxidant activity

The antioxidant activity of the *Solanum xanthocarpum* was assessed using FRAP and reducing power scavenging assays. The FRAP assay evaluates the ability of a substance to reduce Fe³⁺ to Fe²⁺, which is measured by the formation of a coloured complex with TPTZ that can be read spectrophotometrically at 593 nm. Since the antioxidant activity of a substance is usually correlated to its reducing capacity, this assay provides a reliable method to evaluate the antioxidant activity.^[34] The hydroethanolic extract of the herb was found to be having mild anti-oxidant activity.

These results may also be helpful to describe the various pharmacological activities like anti-infective, protective activities.

Anthelmintic activity

In this study we have evaluated the effect of *Solanum xanthocarpum* whole plant extracts on earthworms. The extract showed significant wormicidal activity. Earthworms have the ability to move by ciliary movement. The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy, enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis followed by death of parasite. On introduction of extract to the worms there was slight excitatory activity was observed but as the moment passes the worms were got fatigue and ultimately paralysed leading to there death. In the present study it was observed that the ethanolic extract was

having more potent anthelmintic property than aqueous and hydroethanolic extract. 100mg/ml was having more potential against the worms (Table 3).

The predominant effect of Piperazine citrate on worm is to cause a flaccid paralysis which results in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis.^[46] The crude extracts of *L.siceraria* not only demonstrated paralysis but also caused death of worms especially at higher concentration of 100 mg/ml in nearly same time as compared to reference drug Piperazine citrate.

CONCLUSION

From this preliminary investigation it has been concluded that the herb *Solanum xanthocarpum* is having significant antimicrobial, anti-oxidant activity and anthelmintic activity, the constituent present in the herb might be a responsible for this activity. Further research is in necessary to isolate the compound responsible for this activity.

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