Vol 9|Issue 2| 2019 | 43-48.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF CENTRAL NERVOUS SYSTEM ACTIVITY OF HYDROETHANOLIC EXTRACT OF *SOLANUM XANTHOCARPUM*

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ABSTRACT

Herbal products are extensively used globally for the treatment of many diseases where allopathic fails or has severe side effects. Psycho neural drugs are also have very serious side effects like physical dependence, tolerance, deterioration of cognitive function and effect on respiratory, digestive and immune system. So in his contest the treatment through natural source is seen with the hope that they have the lesser side effects than that observed with synthetic drugs.

Keywords: *Solanum xanthocarpum*, Anti-oxidant, Herbal Products, Anthelmintic, Anti-microbial.

INTRODUCTION

Recent developments in biomedics have pointed to the involvement of free radicals in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging[1-4]. Although living organisms possess enzymatic and non-enzymatic defense systems against excessive production of free radicals, different external factors (smoke, diet, alcohol, some drugs) and aging, decrease the efficiency of such protecting system, resulting in the disturbance of the redox equilibrium established under healthy conditions[5,6]. Antioxidants that can neutralize free radicals may therefore be used to protect the human body from diseases and retard rancidity in foods consumed by humans[7,8]. It is believed that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular diseases and cancer[9-10]. Several research studies have demonstrated that herbal plants contain diverse classes of compounds such as polyphenols, alkaloids, tannins and carotenoids[11,12]. Some of these properties have been related to the action of these compounds as antioxidants, free radical scavengers, quenchers of singlet and triplet oxygen and inhibitors of peroxidation. These phytochemicals are found distributed in different parts of plants[13-15]. There is therefore an increasing interest in finding natural herbal plants that exhibit anti-oxidative activity.Parasitic helminthes affect animals and man, causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites[16]. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases[17]. Hence there is an increasing demand towards natural anthelmintic.

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MATERIAL AND METHODS

Plant Material

The fresh whole plant of *Solanum xanthocarpum* was collected from the rural area. The collected plant was then identified and authenticated by Dr. (Mrs.) Sattarupa Rao, Professor & 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 then ground into fine powder. The powder was sieved to have a uniform size. 100 gm of dried powder was extracted with 400 ml of mixture of ethanol and water (1:1) for 24 hrs. The extraction procedure was repeated trice in order to have optimum extraction. The extract was filtered using a muslin cloth and concentrated at 40±5°C; dried extract was refrigerated at 4°C until use.

Phytochemical Analysis

The phytochemical analysis of the plant was carried out by the standard methods provided by Odebiyi and Ramstard (1978) and Waterman (1993)[18,19].

Test for Tannins

(a) 1 ml of freshly prepared 10% KOH was added to 1 ml of the extract and observed for dirty white precipitate.
(b) 2 drops of 5% $FeCl_3$ was added to 1 ml of the extract and observed for green precipitate.

Test for Saponins (Frothing test)

2 ml of extract in a test tube was vigorously shaken for two minutes and observed for persistent foaming.

Test for Flavonoids

To 3 ml of extract was added 1 ml NaOH and observed for yellow coloration.

Salkwoski's for test Steroids

5 drops of concentrated H_2SO_4 was added to 1 ml of extract and observed for red coloration.

Fehling's test for Glycosides

10 ml of 50% H_2SO_4 was added to 1 ml of the extract in a test tube. The mixture was heated in a boiling water-bath for 15 minutes. 10 ml of fehling's solution was added and the mixture was boiled and observed for brick red precipitate.

Test for Alkaloids

1 ml of HCl was added to 3 ml of extract in a test tube. The mixture was heated for 20 minutes, cooled and filtered. 2 drops of Wagner's reagent was added to 1 ml of the filtrate and observed for reddish brown precipitate.

Test for Carbohydrates

Molisch's test: Extract treated with Molisch reagent (α-napthol in 95% ethanol) and few drops of concentrated H_2SO_4 were added at the sides of the test tube, violet ring appears at the junction indicates the presences of carbohydrates.

Test for Proteins

Millons's test: Extract treated with Millon's reagent (Mercuric nitrate in nitric acid), red color indicates the presence of proteins.

Determination of Anthelmintic Property

Standard

Piperazine citrate (10 mg/ml) was used as 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 out as per the reported method of Ajaiyeoba *et al.*, (2001) with necessary modifications[20]. Different concentrations of extracts (25, 50, and 100 mg/ml in distilled water) were prepared, and three worms (same type) 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).

Determination of Anti-Oxidant Property

Chemicals

Safranine, potassium ferricyanide, trichloroacetic acid, ferric chloride and 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 according to Benzie and Strain (1996) with minor modifications. The FRAP reagent contained 10 mM of 2,4,6-tri (2-pyridyl)-1,3,5-triazine (TPTZ) solution in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$, and acetate buffer (300 mM, pH 3.6) (1:1:10, v/v/v). A 100 µl 50% aqueous methanol of the test compounds was added to 3 ml of the FRAP reagent, and the absorbance was measured at 593 nm after incubation at room temperature for 6 minutes, 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 experiment was performed in triplicate.

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, *Bacillus subtilis* MTCC 1790, *Staphylococcus aureus* MTTC 3160,

Klebsiella oxytoca MTTC 3030, *Candida albicans* MTCC 183.

Preparation of Inoculums

All the microorganisms mentioned above were incubated at 37±0.1ºC, for 24 hrs in Nutrient broth and *C. albicans* in YEPD broth at 28±0.1ºC for 48 hrs.

Antimicrobial Activity

Nutrient agar and YEPD agar (20 ml) were poured into each sterilized Petri dish (10 X 100 mm diameter) after injecting cultures (100µl) of bacteria and yeast and distributing medium in Petri dishes homogeneously. For the investigation of the antibacterial and anticandidal activity, the dried extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 20% and sterilized by filtration through a 0.22 µm membrane filter[22,23]. 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 hrs. At the end of the incubation period, inhibition zones formed on the medium were evaluated in mm.

Study was performed in triplicate and the inhibition zones were compared with those of reference discs. Amphotericin B (10µg) and tetracycline (30 µg) were taken as reference.

RESULTS AND DISCUSSION

Phytochemical Analysis

Phytochemical screening of the extract of *Solanum xanthocarpum* reveals the presence of alkaloids, glycosides, saponins, flavonoids, tannins, phytosterols, steroids, carbohydrates and proteins. Presence of these constituents was used as the indicatives for the pharmacological activity in the extract. Tannins present in the extract may be having anthelmintic property. They can precipitate the protein in the cell membrane thus damaging the outermost membrane allowing release of content and death of the worm. Saponins may exhibit either antioxidant activity or antimicrobial activity or both.

Table 1: Phytochemical Analysis of the Extract of *S. xanthocarpum*.

Sr. No.	Active Principle	Extract
1	Saponins	+
2	Tannins	+
3	Flavonoids	+
4	Steroids	+
5	Glycosides	+
6	Alkaloids	+
7	Carbohydrates	+
8	Proteins	+

Anthelmintic Activity

Helminthes infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintics.

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 it 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. 100 mg/ml extract of *Solanum xanthocarpum* worked very rapidly, it required only less time (9.94±0.3) to paralyse the worms also within 28 minutes it had killed the worms. The time required to paralyse the worms by piperazine citrate was 8.01±0.4 minutes and to kill them was 14.5±0.4 minutes. 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 paralyzed leading to their death. In the present study it was observed that the extract in the dose of 100 mg/ml was having more potential against the worms (Figure 1).

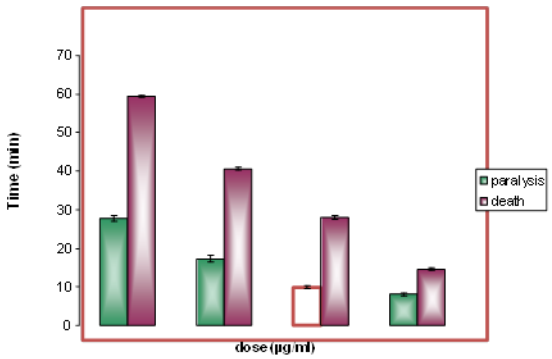

Figure 1. Anthelmintic activity of *S. xanthocarpum*

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. The extracts of *Solanum xanthocarpum* 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.

Antioxidant Activity

Free radical and reactive oxygen species are well known inducers of cellular and tissue pathogenesis leading to several human diseases, such as cancer, inflammatory disorders and diabetes mellitus, as well as in the aging process. Many plant species with antioxidant activities acts as protective agents against these diseases.

In present study, potent antioxidant activity was observed using ferric reducing antioxidant potential (FRAP) assay, scavenging of hydrogen peroxide and total antioxidant capacity for the extract of *Solanum xanthocarpum*. However, the efficacy of extract against various free radicals depending on the specific assay methodology, reflecting the complexity of the mechanism and diversity of the chemical nature of phytoconstituents present.

The FRAP assay evaluates the ability of a substance to reduce Fe^{3+} to Fe^{2+}, which is measured by the formation of a colored complex with TPTZ that can be measured 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. The extract of the herb was found to be having mild anti-oxidant activity (Figure 2).

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

Antimicrobial 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. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants.

The antimicrobial activity of extract of *S. xanthocarpum* was tested under *in vitro* conditions by agar well diffusion method against bacterial and fungal pathogen. The experiment was performed in triplicates. The zone of inhibition of microbial growth with extract is given in Table 2. The extract of *S. xanthocarpum* (50 µg/ml) inhibited the growth of Gram negative bacteria S. *aureus* (14.0±1.0 mm) and *E. coli* (7.3±0.5 mm). Extract of *S. xanthocarpum* (50µg/ml) exhibited significant inhibition over the growth of tested bacterial pathogens except for *Candida albicans* (6.2±0.5 mm) it had given very mild inhibition when compared with amphotericine B (17.3±0.5). For *K. oxytoca* (7.5±0.5 mm) considerable effect was observed when compared with tetracycline (8.3±0.5 mm).It was found that it is not effective against *Bacillus subtilis* results are given in Table 2. It has been reported that the antibacterial activity depends on the total saponins and tannins content of the plant extract [20-23]. The phytochemical study showed the presence of saponins and tannins thus, it can be assumed that the activity was due to saponins and tannins.

CONCLUSION

In the phytochemical screening of the extract of *Solanum xanthocarpum* reveals the presence of alkaloids, glycosides, saponins, flavonoids, tannins, phytosterols, steroids, carbohydrates and proteins. The thin layer chromatography (TLC) confirmed the presence of phenolic constituent and amino acids. Anthelmintic activity of the *Solanum xanthocarpum* extract might be due to the presence of tannins. Likewise antioxidant and anti-microbial activity might be due to the presence of saponins in the extract. Detailed study is necessary in order to get benefit from the herb.

Table 2. *In vitro* Antimicrobial Activities of Extract of *S. xanthocarpum*

Test organism	**Diameter of zone of inhibition (mm)**		
	Hydro alcoholic extract	**Amphotericin B**	**Tetracycline**
S.aureus	14.0±1.0 *	--	20.3±0.5
Bacillus subtilis	--	--	8.6±0.5
Escherichia coli	7.3±0.5**	--	7.6±0.5
Klebsiella oxytoca	7.5±0.5***	--	8.3±0.5
Candida albicans	6.2±0.5*	17.3±0.5	--

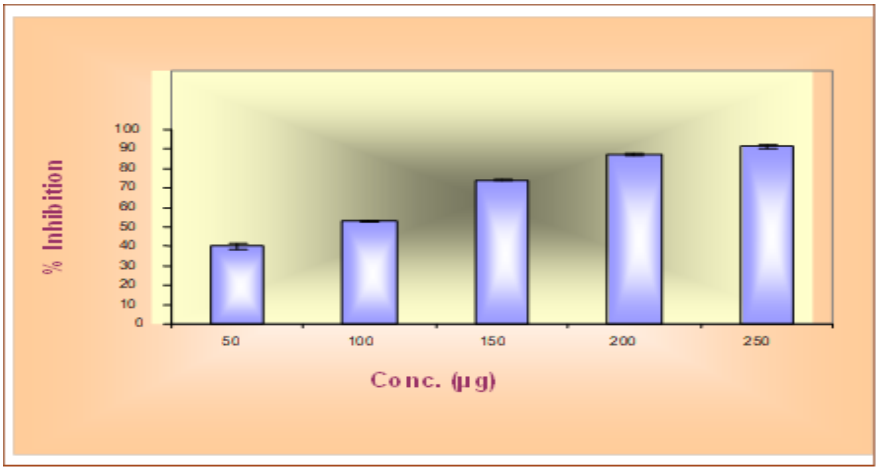

Figure 2: Antioxidant activity of *S. xanthocarpum*

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