Research Article

Evaluation of Anthelmintic and Antioxidant Potential of *Solanum melongena linn.* Leaf Extract using *in-vitro* Models and Estimation of Total Flavonoid Content

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ABSTRACT

The objective of this study is to study the anthelmintic and antioxidant activity of the leaf extracts of *Solanum melongena Linn*. (Family: Solanaceae) and to estimate the total flavonoid content. The aqueous (AE) and ethanolic (EE) leaf extracts of *Solanum melongena Linn*. were prepared and these were evaluated for total flavonoid content by aluminium chloride colorimetric method and for determination of anthelmintic activity using Indian earth worm *Pheretima posthuma*. Various extract concentrations i.e. 10, 20, 40, 80 and 100 mg/mL will be made for this study. The extracts were also evaluated for antioxidant activity by superoxide scavenging activity. Anthelmintic activity was showed by all the extracts at concentration of 10 mg/mL. The ethanolic extract of *Solanum melongena Linn* leaf of 100 mg/mL has exhibited a significant effect ($P<0.001$) when compared to aqueous extract. The antioxidant activity of the extracts was quite prominent but it was comparatively lower when compared to the standard significance ($P<0.05$). Total 5.39 mg QE/100 g flavonoid traces observed in the extract. The above analytical study shows that the *Solanum melongena Linn* leaf extract has shown a good anthelmintic and antioxidant activity.

Keywords: Helminthiasis, *Solanum melongena Linn.*, Aluminium chloride colorimetric method, *Pheretima posthuma*.

INTRODUCTION

Helminthic diseases now a day is the major health related concern in all over the world. They affect a large human population in endemic areas and hence can cause life threatening issues. WHO reported that a big number of people around the world suffer from parasitic worm infections[1]. Worms like pinworms, round worms and tapeworms are common parasites which infected the human body parts and responsible for Helminthiasis disease. These worms often reside in gastro intestinal tract as well as target the liver and other organs of human body. Helminth eggs are excreted by infected humans through their faeces and these eggs can contaminate the soil[2]. Drugs that completely kill or remove the infesting worms are termed as anthelmintic drugs. Unfortunately some common side effects like nausea, vomiting, abdominal pain, hair loss, fall in blood pressure decreased, sedation, fever, and body ache are associated with the popular drugs which are available to target these worms[3]. Considering the above problems, it is required do identify new alternative therapies for this helminthes. Plant based herbal treatment will be surely beneficial in this area. Therefore, herbal plant extract based anthelmintic drug development was focused in this research work.

Plants are used by mankind as herbal remedies for several diseases from ancient times. In India Ayurveda, Unani and Sidha systems of medicines are broadly used to treat and curing of many diseases. *Solanum melongena* was significantly used as an effective therapy against various human diseases conditions from the centuries. "Melongene" is the common name of this plant. In most of the regions of Asia, it is called brinjal. In America, Australia and Canada it is known as "eggplant". In Britain and sometimes in Canada it is called "aubergine"[4]. The fruit of this plant is very popular in India for used as vegetable. *Solanum melongena* exhibits many traditional uses and also reported for showing various potent pharmacological actions[5]. Recently the insecticidal activity of *Solanum melongena* is reported, which is of great importance. Ethanolic extract of *Solanum melongena* leaf showed very prominent insecticidal activity against *Sitophylus oryzae*, Carpenter ant Pantry weevil larvae[6].

MATERIALS AND METHODS

Plant material

The fresh leaves of *Solanum melongena* were procured from the rural agricultural land area of Chhattisgarh, India and were authenticated.

Preparation of extract

Leaves of *Solanum melongena* were dried in shade and coarsely powdered. Then this powder was subjected to Soxhlet extraction by water and ethanol using as a solvent for 72 hrs. The extracts were then subjected to distillation for removing the solvent and then the concentrated mass was dried on water bath for further evaporation.

Drugs and chemicals

The XOD (xanthine oxidase), NBT (nitro blue tetrazolium), SOD (superoxide dismutase) were purchased from Sigma Chemical Company, Albendazole (Alkem Laboratories Ltd.) and rest of all chemicals used in the study are of analytical grade.

Preliminary phytochemical screening

To evaluate the presence of various active constituents, the aqueous and ethanolic extracts of *Solanum melongena* leaves are subjected to preliminary phytochemical screening[7].

Estimation of total flavonoid content

Aluminium chloride colorimetric assay was used to estimate the amount of flavonoids that are present in the extracts[8]. About 1 mL of extracts or quercetin standard solutions of different concentrations i.e. 20, 40, 60, 80 and 100 µg/ mL was added to a volumetric flask (10 mL), which prior contains 4 mL of distilled water. Then 0.3 mL of $NaNO_2$ (5%) was added in to this flask and then 0.3 mL of $AlCl_3$ (10%) was mixed after 5 min. Then 2 mL of 1 mol/L NaOH was added after 5 min and the volume was made upto 10 mL with distilled water. At last the above solution was properly mixed and then this solution was subjected to the measurement of absorbance at 510 nm against the blank. The quantity of flavonoids present in the extracts was denoted as mg quercetin equivalents (QE).

Determination of anti-oxidant property by superoxide scavenging activity

For the analysis of superoxide scavenging potential, the method was adopted as described by Murakami et al., 1996 and modified according to the need. The principle of this method was generation of superoxide by oxidation of xanthine to uric acid with the help of XOD. This generated superoxide is then reacts with NBT and a colour change occurs from light yellow to dark purple. This colour change was then measured by spectrophotometer at 560 nm. The superoxide radicals scavenging ability of sample can be determined by inhibition of NBT reduction. Methanol extracts of samples were N_2 air-dried and dissolved in water before the assay. In a 20mL tube, 20 µL of water or methanol extract, 500 µL of PBS (pH7.2) containing 0.24mM NBT and 0.4mM XA was added, followed by 500 µL of a XOD solution (0.049 U/mL) in PBS. After incubation at 37˚C for exactly 20 min, the reaction was stopped by adding 1mL of a sodium dodecyl sulphate solution (69mM). Visible absorption of the reaction mixture at 560nm was measured. It is said to be 100% of NBT reduction inhibition, when the difference between the absorbance of the reaction with and without XOD occurs. The interference of the sample colour to readings was determined by using a blank sample without NBT[9,10].

Experimental model

Pheretima posthuma (Indian earth worms) resembles anatomically as well as physiologically with intestinal roundworm parasite of the human and therefore they used to analyzed anthelmintic properties[11,12]. These earth worms are very common and the adult ones are collected from the moist land soil and then cleaned with normal saline to wash out all noxious matter.

Antihelmintic activity

In this analysis, Indian adult earth worms were used which having 4-5 cm. length and 0.1-0.2 cm. width. These worms are divided into groups and each group contains six earthworms. Aqueous and ethanolic extracts were prepared of different concentrations i.e.10, 20, 40, 80, and 100 mg/mL. Albendazole as standard (10 mg/mL in 50 mL of water), and the earth worms were placed and observed for anthelmintic potential. If worms are not revived when placed in normal saline it means they are paralyzed. When there is no evidence of motility of the worms including colour fading of the body, it means worms are dead. Time of paralysis and death of individual worm was observed[13,14].

Statistical analysis

All results are expressed as mean ±SD, and the data was calculated by using ANOVA (analysis of variance) followed by Dunnets test. Values would be considered statistically significant when *P* value was less than 0.001.

RESULTS

Preliminary phytochemical screening

Results from the phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins and proteins in both aqueous and ethanolic extracts.

Estimation of total flavonoid content

The flavonoid contents were present in aqueous and ethanolic extracts of *Solanum melongena Linn* leaves, and the total flavonoid content was found to be 5.39 mg QE/100 g.

Determination of anti-oxidant property by superoxide scavenging activity

Superoxide radical is considered a major biological source of reactive oxygen species[15]. Superoxide anion facilitates the forming of strong and harmful hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress[16]. The equal doses of extracts and ascorbic acid were used to evaluate superoxide radical scavenging potential, which was found in between 25 - 500µg/ml. When compared to ascorbic acid, the super-oxide scavenging activity of the *Solanum melongena Linn* leaf extract was found below ($P < 0.05$). This study revealed that the extract can be used as a prominent superoxide anion scavenger, further it can be utilized therapeutically to control oxidative stress.

Anthelmintic activity

The aqueous extract causes paralysis of worms. Its activity was depending upon the dose ranging from loss of motility to loss of response to external stimuli, which eventually leads to death. 100 mg/mL dose has shown the significant value $P<0.001$ when compared with standard drug albendazole (10 mg/mL). The ethanolic extract has taken the time of 38.64±2.24 min for paralysis and time of death was 88 .00±1.46 min, and the aqueous extract has taken

the time of 65.00±2.23 min for paralysis and time of death was 151.20±2.32 min. The results showed that the ethanolic extract showed better activity than that of aqueous extract (Table 1).

Table 1: Anthelmintic activity of *Solanum melongena Linn* leaf extracts on *P. posthuma*.

Groups	Drug treatment	Concentration (mg/mL)	Time for paralysis (min)	Time for death (min)
I	Albendazole	10	34.25±3.20	45.33±2.20
II	AE	10	87.65±5.44**	184.76±5.32***
III	AE	20	82.65±3.20**	174.00±2.89***
IV	AE	40	74.35±2.36**	163.75±3.20***
V	AE	80	71.26±2.46***	157.28±2.26***
VI	AE	100	65.00±2.23***	151.20±2.32***
VII	EE	10	54.10±2.68**	103.00±2.20***
VIII	EE	20	51.00±2.20**	100.46±1.38***
IX	EE	40	47.00±1.64***	97.42±1.76***
X	EE	80	44.20±2.10***	92.68±1.20***
XI	EE	100	38.64±2.24***	88 .00±1.46***

Values are expressed as mean±SD. The results were analyzed by ANOVA (analysis of variance) followed by Dunnets *t*-test. P<0.001 when compared with Group II. AE: Aqueous extract; EE: Ethanolic extract.

DISCUSSION

The extracts of *Solanum melongena Linn* leaf (both aqueous and ethanolic) were used to evaluate anthelmintic activity, which has shown the dose dependent activity. The mean±SD values were calculated for each *Solanum melongena Linn* leaf extract. The result of anthelmintic activity on earthworm *P. posthuma* reveals that the different concentrations used for aqueous and ethanolic extracts of *Solanum melongena Linn* have shown paralysis and death of earthworms, which were compared with a reference drug albendazole. β-tubulin is the target site for albendazole. It inhibits its polymerization followed by interference with microtubule dependent functions like glucose uptake[3].

From phytochemical screening, the aqueous and ethanolic leaf extracts of *Solanum melongena Linn* showed the presence of alkaloids, flavonoids, tannins, proteins and saponins, which could have been responsible for antihelmintic potential[17]. Anthelmintic activity of the leaf extract may attribute to the presence of alkaloids and tannins. Possible mechanism of anthelmintic activity of *Solanum melongena Linn* leaf extract may be due to tannins which bind to free proteins in the gastrointestinal tract of infested animal or it can bind to glycoprotein on the cuticle of the parasite and leading to death of the parasite[18]. Another possibility is due to alkaloids, which can target central nervous system and cause paralysis of the *P. posthuma* worms[19].

The study of anti-oxidant activity by superoxide scavenging concludes that the extract of *Solanum melongena Linn* leaf is an effective and potent superoxide anion scavenger that may be used therapeutically against oxidative stress. From the estimation study of total flavonoid content in aqueous and ethanolic extracts of *Solanum melongena Linn* leaves, the results reveeled that the flavonoid contents were present in the extract remarkably.

CONCLUSION

The aqueous and ethanolic leaf extracts of *Solanum melongena Linn* leaves were prepared and evaluated for their anthelmintic as well as anti-oxidant potential by superoxide scavenging estimation. The extracts were also placed for phytochemical screening and examined for total flavonoid content. From the above studies, results were concluded that the aqueous and ethanolic extracts of *Solanum melongena Linn* leaf have shown a prominent anthelmintic and antioxidant activity, and further *in vivo* studies are needed to be performed to evaluate the therapeutic effectiveness of the extracts which can be used as anthelmintic drug.

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