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Phytochemical analysis and anthelmintic activity of extracts of *Solanum Xanthocarpum*

Dr.K.Subharani Devi
Department of chemistry, Standard College, Kongba.

ABSTRACT
Crude methanol extract and subsequent solvent fractions of *Solanum* Xanthocarpum were evaluated for anthelmintic activity against sheep intestinal worms *Haemonchus contortus.* The extracts were also evaluated for total phenolic and total flavonoid contents using colorimetric methods. The ethylacetate extract showed significant anthelmintic effect with high death rate of worms at hourly interval at a concentration of 0.05 mg/ml. Total phenolic content in the crude methanolic extract was 342 ± 2.84 mg TAE/g dE. After fractionation the maximum concentration of phenols was measured in ethylacetate fraction (426 ± 3.87 mg TAE/g dE). Total flavonoid content in the crude methanol extract was 128 ± 2.34 mg QE/g dE. After fractionation highest concentration of flavonoids was measured in ethylacetate fraction (180 ± 2.51 mg QE/g dE). So the results indicated that the polar fractions of *Solanum* Xanthocarpum containing high concentration of phenolics and flavonoids possess high anthelmintic activity.

Key words: *Helminthiasis, anthelmintic activity, phenolics, flavonoids, Solanum Xanthocarpum.*

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1. INTRODUCTION

Ruthless morbidity affecting principally population in endemic areas cause by parasitic diseases. Helminthiasis is a widespread parasitic infection among humans and animals caused by helminths. The disease is highly prevalent particularly in developing countries due to inadequate sanitary conditions and poor management practices. Helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, pneumonia, eosinophilia and anemia. Anthelmintics are drugs used to expel parasitic worms from the body by paralyzing or killing them. It has importance in humans and veterinary medicines. The gastrointestinal helminthes have become resistant to currently available anthelmintic drugs causing problem in treatment of helminthes diseases (Sondhi & Shahu, 1994). Hence there is an increasing demand towards natural anthelmintics.

A large number of medicinal plants have been used for the treatment of helminthiasis in humans and animals (Chopra *et al*., 1956, 1958; Akhtar, 2000). Solanaceae is a family of flowering plants that includes a number of important agricultural crops, although many species are toxic plants. Many plants of the family are used by humans, and are important sources of food, spicesand medicine.
Medicinally, as well as in terms of poisoning and psychotropic effects, members of Solanaceae have been valued for their alkaloid content and used throughout history. The plants of family Solanaceae also have the properties of anti-malarial activities (Ramazani *et al*., 2010) and also used for the treatment of cold, eye diseases and heart pains. The purpose of the present study was to evaluate the crude methanolic extract and subsequent solvent fractions of *Solanum Xanthocaepum* for anthelmintic activity. The extracts were also evaluated for total phenolic and flavonoid contents. The study can be of significant importance in developing cheaper and easily available anthelmintics with lesser side effects.

Chemicals
n-Hexane, dichloromethane, ethyl acetate, acetone and methanol were all of analytical grade, purchased from Fischer Scientific. For purity measures, the chemicals were used after re- distillation. Aluminium chloride ($AlCl_3$), potassium hydroxide (KOH), ferric chloride ($FeCl_3$), sulphuric acid (H_2SO_4), Dragendroff reagent, sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), Folin-Ciocalteu reagent, quercetin and tannic acid were purchased from Sigma Aldrich. Sodium chloride (NaCl), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were purchased from RDHLaborchemikalien.

Equipments
Plant extracts were concentrated with rotary evaporator. All UV-Vis spectral analyses were carried out in methanol with UV-Vis double beam spectrophotometer (Hitachi, U2800). The sterilization of phosphate buffer solution was done in autoclave.

Extraction
The fresh plant material (360 g) was washed and dried in shade. The dried and powdered material (70 g) was extracted in redistilled methanol for seven days at room temperature away from sunlight, with occasional stirring. The soaking mixture was filtered to isolate the methanol extract. The process was repeated thrice and the combined extracts were concentrated under reduced pressure in the rotary evaporator to give crude methanol extract. For liquid- liquid partition the crude methanol extract was dissolved and suspended in double distilled water and filtered through filter paper. The water extract was partitioned between *n*-hexane, dichloromethane, ethyl acetate and acetone. The process afforded non-polar fractions of *n*-hexane and dichloromethane and polar fractions of ethyl acetate, acetone and water. The water fraction was freeze-dried to give water extracts. All the extracts were weighed (Table 1) and stored in tightly sealed dark glass containers at 4ºC for further analysis.

Qualitative Phytochemical Analysis
For qualitative phytochemical analysis standardchemical methods were performed (Harborne, 1973).
Glycosides: For glycosides 1 mL of freshly prepared 10% KOH was added to 1 mL of extract. The presence of glycosides was confirmed by the formation of brick red precipitates.
Saponins: For saponins, frothing test was performed in which 2 ml of the extract was vigorously shaken in the test tube for 2 minutes.Presence of frothing indicated saponins.
***Steroids*:** Steroids were identified by adding 5 drops of concentrated H_2SO_4 to 1 mL of the extract in a test tube. Red coloration indicated the presenceof steroids.
Triterpenes: For triterpenes, 5 drops of concentrated H_2SO_4 were added to 1 mL of extract. Appearance of blue green colour indicated the presence of triterpenes.
Flavonoids: Presence of flavonoids was tested by adding 1 mL of freshly prepared 5% $AlCl_3$solution to 1 mL of extract. Yellow coloration indicated the presence of flavonoids.
Phenolics: For phenolics, two drops of 5% $FeCl_3$ were added to 1 mL of the extract in a test tube. Presence of greenish precipitate indicated the presence of phenolics.
Alkaloids: To detect the presence of alkaloids 0.2 gm of plant extract was warmed with 2% sulphuric acid in a test tube for 2 minutes. The mixture was filtered in a separate test tube and few drops of Dragendroff reagent were added and observed for the presence of orange red precipitates for the presence of alkaloids.

Quantitative Phytochemical Analysis
The crude extract and solvent fractions of *S. Xanthocarpum* were analyzed quantitatively for total phenolic and total flavonoid content using standard methods.

Determination of total phenolics
Total phenolic contents were determined by using Folin-Ciocalteu (FC) reagent (Cliffe *et al*., 1994). For analysis 20 µL of plant extract was mixed with 1.58 mL of deionized water and 100 µL of FC reagent and incubated for 10 min at room temperature. To the reaction mixture 300 µL of 25% Na_2CO_3 solution (w/v) was added and again incubated at 40ºC. After cooling for 0.5 h, absorbance was measured at 765 nm against the blank (containing 20 µL of extracting solvent instead of plant sample). TPC of the sample was determined with a linear equation based on the standard calibration curve prepared under the same conditions using tannic acid as standard (Figure 1). The results were expressed as mg tannic acid equivalent (TAE)/g dry extract (dE).

$Y = 2.807x + 0.026$; $r^2 = 0.914$
(where *Y* is the absorbance and *x* is the concentration of tannic acid mg/ml).

Determination of total flavonoids
Total flavonoid content (TFC) was determined by using aluminium chloride colorimetric method (Dewanto *et al*., 2002). For analysis 250 µL of the extract was diluted with 500 µL of deionized water and 90 µL of 5 % (w/v) $NaNO_2$ solution was added and left to stand for 6 min. Then, 180 µL of 10% (w/v) $AlCl_3$ solution was added to the above mixture and allowed to stand for another 5 min followed by the addition of 600 µL of 1 M NaOH solution. The final volume was made up to 3 mL with deionized water. Absorbance was measured at 510 nm against blank (250 µL of plant extract was replaced by 250 µL of extracting solvent). TFC was calculated from linear equation based the calibration curve of quercetin, used as standard, obtained under same experimental conditions as described above (Figure 2). The results were expressed as mgquercetin equivalent (QE)/g dE.

$Y = 0.131 x + 0.016$; $r^2 = 0.918$
(where *Y* is the absorbance and *x* is the concentration of quercetin in mg/ml).

Anthelmintic Activity Preparation of Test Solution

Test solution was prepared by dissolving 0.5 mg of dried plant extract in 0.1 ml of DMSO and 9.9 ml of Phosphate Buffer Solution (PBS) to make the final volume 10 mL (0.05 mg/ml).

Anthelmintic activity of the plant extracts was examined by using the method of Ajaiyeoba *et al*. (2001). The assay was carried out on intestinal parasite of sheep *Haemonchus contortus* which resembles with intestinal worms of human beings. The worms were obtained from intestine (abomasi) of freshly slaughtered sheep. Intestine (abomasi) of sheep were collected from the local slaughter house and washed with normal saline solution to remove all the faecal matter. The intestines were then dissected and worms were collected and kept in normal saline solution. The average size of these worms was 1-2 cm.

The abomasi of the freshly slaughtered sheep were dissected and worms collected in a dish. The worms were washed and suspended in the PBS at room temperature. Ten worms per petri dish were used to study the effect of plant extracts. The experiments were performed in triplicates. The motility was recorded with hourly interval for 6 h. Finally the treated worms were kept for 30 minutes in the lukewarm fresh PBS to observe the revival of motility.

2. RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

The preliminary phytochemical analysis of different plant extracts evidenced the presence of multiple components in the extracts. The results revealed the presence of flavonoids, glycosides, tannins, steroids, saponins, terpenes, and phenolic compounds (Table 2).

Total phenolic content

The results of analysis of TPC are summarized in Figure 3. TPC in the crude methanolic extract was 342 ± 2.84 mg TAE/ g dE. After fractionation the highest concentration of phenols was measured in ethyl acetate fraction (426 ± 3.87 mg TAE/g dE) followed by dichloromethane fraction (416 ± 3.52 mg TAE/ g dE). The minimum amount of phenolics was determined in *n*-hexane fraction (228 ± 2.64 mg TAE/g dE).

Total flavonoid content

The results of analysis of TFC are summarized in Figure 3. TFC in the crude methanolic extract was 128 ± 2.34 mg QE/g dE. After fractionation highest concentration of flavonoids was measured in ethyl acetate fraction (180 ± 2.51 mg QE/g dE) followed by dichloromethane fraction (140 ± 2.30 mg QE/g dE). The minimum amount of flavonoids was determined in *n*-hexane fraction (44 ± 1.00 mg QE/g dE). High solubility of flavonoids in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction.

Anthelmintic activity

The results of anthelmintic activity of all the plant samples are summarized in Table 3 and Figure 4. All the extracts showed different anthelmintic activity in terms of mortality rate at the same concentration during the six hours time period. This difference in activity is attributed to difference in type and quantity of different phytochemicals present in each plant extract.

Several plants of family Solanaceae and particularly from the genus *Solanum* have been shown to possess anthelmintic activity (Gunaselvi *et al*., 2010; Mathri *et al*., 2011; Yadav & Tangpu, 2012). In all these studies mostly the crude methanol or ethanol extracts and aqueous extracts were tested for anthelmintic activity. In the present study the crude methanol extract was further partitioned using solvents of various polarity and the fractions were tested for anthelmintic activity. Among the extracts the ethyl acetate, acetone and aqueous fractions showed significant anthelmintic effect with high death rate in the given time interval at the tested concentration (0.05 mg/ml). Levamisole used as positive control showed 100% mortality rate after 3 h. The results can be considered significant since the extracts are crude samples with a number of compounds and can be a source of phytochemicals with anthelmintic activity comparable to standard drugs used. Although the rate of death of worms after each hour was different for each fraction, at the end of six hour time period the rate of death of worms was same. The effect of extracts on the death of the worms, according to the result may be indicated as ethyl acetate > acetone > aqueous > dichloromethane > crude methanolic > *n*- hexane extracts. In particular ethyl acetate extract exhibited an increased death of worms at hourlyinterval.

A number of studies are available for anthelmintic activity of tannins, alkaloids and flavonoids (Anthnasiadou *et al*., 2001; da Silva *et al*., 2008; Wang *et al*., 2010;). The presence of these phytochemicals may be responsible for the observed anthelmintic activity of plant extracts in present study. Tannins have been shown to interfere with coupled oxidative phosphorylation thus blocking ATP synthesis in these parasites (Martin, 1997). Tannins may also bind to the cuticle of the helminth's body surface making it immobile causing the parasite to become paralysed leading to its death (Thompson & Geary, 1995). Presence of tannins in ethyl acetate, acetone and aqueous fractions may be responsible for high anthelmintic activity of these extracts.

The difference in activity of different plant extracts may also be due the difference in total phenolic and flavonoid contents in these extracts. Since the polar fractions contained a high phenolic and flavonoid content than the non-polar fractions this may explain the observed difference in the anthelmintic activity of these extracts.

3. CONCLUSION

In the present study the crude extracts and fractions of *Solanum Xanthocarpum* were evaluated for anthelmintic activity against sheep intestinal worms
H. contortus and significant activity was observed for the polar fractions. The extracts were also tested for qualitative and quantitative analysis of selected plant metabolites. The extracts were found to be rich both in quality and quantity of metabolites especially phenolics and flavonoids. The study thus concludes that the polar extracts of *S. nigrum* can be used for the treatment of parasitic diseases such as helminthiasis and are a cheap source of phenolics such as alkaloids, tannins and flavonoid with biological activities including antioxidant, antibacterial and antifungal.

Table 1: Amount (g) and % yield of different solvent fractions of *Solanum Xanthocarpum.*

MeOH		***n*-Hexane**		**CH_2Cl_2**		**EtOAc**		**Acetone**		**Aqueous**	
wt. (g)	% age	wt. (g)	% age	wt. (g)	% age	wt. (g)	% age	wt. (g)	% age	wt. (g)	% age
11.4	16.28	2.2	19.3	0.3	2.63	1.2	10.53	2.7	23.68	5.0	43.86

Table 2: Qualitative phytochemical analysis of extracts

Solvent	**Flavonoids**	**Glycosides**	**Tannins**	**Steroids**	**Saponins**	**Terpenes**	**Phenolics**
MeOH	+	—	—	+	—	—	—
n-Hexane	—	—	—	+	—	+	—
CH_2Cl_2	+	—	—	+	+	+	+
EtOAc	+	—	+	—	+	—	+
Acetone	—	+	+	+	—	—	+
Aqueous	+	—	+	+	+	—	—

(+) = indicates presence, (–) = indicates absence

Table 3: Comparison of *in vitro* anthelmintic activity of different extracts of *SolanumXanthocarpum*

Treatments	**Number of worms surviving at hourly interval**						
	0	**1**	**2**	**3**	**4**	**5**	**6**
MeOH	10.00 ± 0.00	9.66 ± 0.57	7.66 ± 0.57	7.33 ± 1.15	6.00 ± 1.00	5.00 ± 1.00	1.66 ± 0.57
n-Hexane	10.00 ± 0.00	9.66 ± 0.57	8.33 ± 0.57	5.66 ± 0.57	5.00 ± 1.00	3.66 ± 0.57	2.33 ± 0.57
CH_2Cl_2	10.00 ± 0.00	9.00 ± 1.00	7.66 ± 0.57	6.33 ± 0.57	5.00 ± 1.00	3.66 ± 0.57	1.00 ± 1.00
EtOAc	10.00 ± 0.00	9.66 ± 0.57	9.00 ± 1.00	8.33 ± 0.57	7.00 ± 1.00	3.66 ± 0.57	0.33 ± 0.57
Acetone	10.00 ± 0.00	9.00 ± 1.00	7.00 ± 1.00	6.00 ± 1.00	5.66 ± 0.57	3.66 ± 0.57	0.33 ± 0.57
Aqueous	10.00 ± 0.00	9.66 ± 0.57	9.00 ± 1.00	7.66 ± 0.57	5.66 ± 0.57	3.66 ± 0.57	0.33 ± 0.57
Levamisole	10.00 ± 0.00	5.00 ± 1.00	3.66 ± 0.57	2.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

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