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Research Article

A Study on *Ardisia solanacea* for Evaluation of Phytochemical and Pharmacological Properties

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ABSTRACT

The present study was conducted to detect possible phytochemicals and evaluate antioxidant, antimicrobial, thrombolytic, anthelmintic and cytotoxic activities of the extract of *Ardisia solanacea*. Phytochemical screening was carried out using the standard test methods of different chemical group. For investigating the antioxidant activity, two complementary test methods namely DPPH free radical scavenging assay and total phenolic content determination were carried out. For the evaluation of in vitro antimicrobial activity, disc diffusion method, and to determine the thrombolytic activity, the method of Prasad et al., 2007 with minor modifications were used. Evaluation of cytotoxic activity was done using the brine shrimp lethality bioassay. The anthelmintic study was carried out by the method of Ajaiyeoba *et al.* with minor modifications. The extracts were a rich source of phytochemicals. In DPPH free radical scavenging test, the petroleum ether soluble fraction showed the highest free radical scavenging activity with IC50 value 40.04µg/ml. while compared to that of the reference standards ascorbic acid . *Ardisia solanacea* was also found as a good source of total phenolic contents. Morever, the extracts revealed moderate antimicrobial activity at the concentration of 400 µg/disc. By comparing with the negative control the mean clot lysis % was significant (p value <0.0009). In cytotoxic activity test, the petroleum ether soluble fraction showed significant cytotoxic potential (LC50 value of 0.703 µg/ml) among all the fractions comparing with that of standard vincristine (0.544 µg/ml). The crude extract of *Ardisia solanacea* produced a significant anthelmintic activity in dose dependent manner and the activity of crude extract was comparable with that of standard drugs. Therefore, further studies are suggested to determine the active compounds responsible for the pharmacological activities of the plant extracts.

Key words: Phytochemicals, antioxidant, antimicrobial, thrombolytic, anthelmintic and cytotoxic.

INTRODUCTION

Nature provides different remedies for human being from its plants, animals and other sources to treat all ailments of mankind[1,2]. Medicinal plants are important contributors among all natural sources[3]. They always play an important role in the development of health in mankind[4]. According to World Health Organization (WHO), about 80% of the world population still uses medicinal plants for different medical purposes[2]. Even in recent time, people are still taking traditional medicine for their primary health care[3]. It has been examined that fruits and herbs containing phyto-constituents and non-nutritive may protect human from different types of diseases for their biological activities[3]. It is now fully believed that phytochemicals obtained from the medicinal plants serve as active molecules in the modern medicines[4]. *Ardisia solanacea* is a species of the genus *Ardisia*[5]. Almost 500 species of *Ardisia* are found throughout tropical and subtropical regions of the world. Several of those species have been used as ornamental plants, medicines and food[5]. *Ardisia solanacea* (*A. solanacea*) is locally known as Banzam, wild berry in English. This plant has stimulant and carminative properties[6]. The plant is applied in the treatment of diarrhea, dysmenorrhea, gout, mental disorder, rheumatic arthritis, skin sore and vertigo. Roots have antibacterial activity. Other species of the *Ardisia* have been reported for their cytotoxic, thrombolytic and antioxidant properties[6].

Antioxidants are molecules which are capable of preventing the oxidation of other molecules. Natural antioxidants are more beneficial for human body than synthetic antioxidants. Plants are rich sources of natural antioxidants[7]. Antimicrobial assay procedures provide a valid measure of antibiotic activity[8]. Antibiotics are important weapons to eliminate bacterial infections[9]. Disc diffusion is a simple and convenient method of antimicrobial activity[8]. Thrombosis is a patho-physiological condition which is responsible for the

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Table 1: Presence of Phytochemicals in *Ardisia solanacea*

S. No.	Phytochemicals	Test	Extract	Result
1	Alkaloids	Wagner's test	Methanolic	+
		Hager's test	Methanolic	+
2.	Carbohydrates	Hager's test	Methanolic	+
		Benedict's test	Methanolic	+
		Fehling's test	Methanolic	+
3	Glycosides	Legal's test	Methanolic	_
4	Saponin	Froth Test	Methanolic	+
5	Phytosterols	Libermann-Burchard's test	Methanolic	+
6	Phenol	Ferric Chloride Test	Methanolic	+
7	Tannins	Gelatin Test	Methanolic	+
8	Flavonoids	Alkaline reagent test	Methanolic	+
		Lead acetate test	Methanolic	+
9	Proteins and amino acids	Xanthoproteic Test	Methanolic	+
		Ninhydrin Test	Methanolic	+
10	Terpenes	Copper acetate Test	Methanolic	_

Key: (+) = Present and (−) = Absent

Table 2: Total Phenolic Content of *A. solanacea* crude extracts and its fractions

Ardisia solanacea	Concentration (µg/ml)	Absorbance at 760nm	Gallic Acid Equivalent(µg/mg)
Methanolic Crude Extract	250	0.301	58.353
Petroleum ether fraction of Crude Extract	250	0.348	69.412
Carbon Tetrachloride soluble fraction of Crude Extract	250	0.212	37.412
Chloroform soluble fraction of Crude Extract	250	0.099	10.824

Table 3: Comparative DPPH radical scavenging activity of different extracts of *A. solanacea* and standards of Ascorbic Acid (AA).

Concentration (µg/ml)	Methanolic Crude Extract	Carbon tetra Chloride Extract	Petroleum Ether Extract	Chloroform Extract	Aquous Extract	Ascorbic Acid (Standard)
500	78.94	66.44	84.92	66.93	64.55	97.43
250	76.12	64.61	80.60	62.57	58.97	83.99
125	70.84	59.08	75.56	51.13	46.22	72.41
62.5	62.48	54.11	67.27	38.56	37.92	65.49
31.25	46.85	39.18	54.71	20.19	23.44	59.25
15.625	20.09	15.42	25.96	12.73	20.47	56.81
7.813	12.73	11.45	13.97	10.80	14.34	54.39
3.90	6.76	8.18	10.34	7.06	11.60	49.49
1.953	3.07	7.65	7.72	6.01	7.78	41.79
IC50 (µg/ml)	52.11	91.34	40.04	157.39	174.13	14.64

development of acute coronary disorders such as strokes and heart attacks. Therefore, the proper choice of thrombolytic agents to reduce platelet aggregation is essential in the management of thrombosis patients[10].

Recently, the thrombolytic activities of some traditional plants have been studied and significant thrombolytic activities were found for some medicinal plants[11]. Now a day, available anti-tumor drugs have been associated with serious side effects. If any significant cytotoxic effect exerting herbal medicine can be obtained which is locally available and relatively cheap then it will be very helpful in the treatment of cancer[12]. Besides, helminthes still cause problems for human and animal. Drugs obtained from plant origin serve as prototype to develop more effective and less toxic anthelmintic medicines[13].

According to our knowledge, no significant study is presently available that document the phytochemical and pharmacological properties of *A. solanacea.* So, in this study, our main goal was to evaluate the phytochemical and pharmacological properties of *A. solanacea* to validate its use in folkloric treatments.

MATERIALS AND METHOD

Plant materials

Table 4: Antimicrobial activity of Methanolic crude extract of *A. solanacea* and its different fractions.

Types of micro-organism		Samples of *A. solanacea* – zone of inhibition mm					
		PEAS	MAS	CTCAS	CAS	AAS	Ciprofloxacin
Gram Positive Bacteria	*Bacillus subtilis*	8.2	12.9	Nil	Nil	10.4	41.1
	Staphylococcus aureus	10.8	9.7	7.8	Nil	9.8	32.3
Gram Negative Bacteria	*Pseudomonas aeruginosa*	Nil	12.7	Nil	Nil	Nil	41.3
	Salmonella typhi	8.9	12.4	Nil	7.4	10.7	36.2
	Escherichia coli	9.4	8.4	8.8	Nil	Nil	32.5
Fungi	*Candida albicans*	Nil	10.9	8.4	Nil	10.4	34.5
	Aspergillus niger	8.1	7.0	Nil	Nil	9.9	45.7

For this present investigation, the *A. solanacea* were collected from Chittagong in December 2011. After collection, the taxonomic identification of the plant was carried out with the help of taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (Accession no. 38305). The voucher specimen was also deposited there for future reference.

Table-5: Results Of % of clot lysis of crude methanolic extract and petroleum ether fraction of *A. solanacea,* Streptokinase and 0.9% Nacl

Sample name	Concentration	% of clot lysis
Methanolic crude extract	5	11.62
	10	9.26
	20	7.2
Petroleum ether fraction	5	17.07
	10	12.03
	20	9.61
Streptokinase as standard	15000 I.U.	26.14
	30000 I.U.	45.35
0.9% saline water as Control		5.57

Chemicals and Reference drug

For performing these experiments , 1,1-Diphenyl-2-picryl hydrazyl (DPPH), Trichloro acetic acid (TCA), L-Ascorbic acid, Butylated Hydroxy Anisole (BHA), Gallic acid, Folin-ciocalteu phenol reagent, phosphate buffer (pH 6.6), distilled water, streptokinase (30000 IU and 15000 IU) of analytical grade (Merck, Darmstadt, Germany) were used. All the chemicals and reagents were purchased from Sigma Chemical Co. Ltd, (St. Louis, MO, USA) and E. Merck (Germany).

Extraction of plant materials

For methanolic extraction 400 gm of air dried and powdered sample were submerged in 2500 ml of 80% methanol (Merck KGaA, Darmstadt, Germany) in clean, sterilized and flat-bottomed glass container. The container and its contents was sealed and kept for maceration for 20 days with occasional shaking and stirring. At the end of 20th day, the whole mixture was filtered using filter cloth and Whatman® filter paper (Sargent-Welch, USA). The resultant filtrate was then allowed to evaporate in water bath maintained 45ºC to dryness and thus a greenish black colored semisolid extract was obtained (yield 25 gms). This gummy concentrate was designated as crude extract of methanol.

Solvent-solvent partitioning

Solvent-solvent partitioning was done using the protocol designed by Kupchan[12] and here this protocol is slightly modified. The crude extract (5 gm) was dissolved in 10% aqueous methanol. It was extracted with Petroleum Ether, then with carbon tetrachloride and finally with Chloroform.

Phytochemical screening

Small quantity of freshly prepared methanolic extract and different fractions of barks of *A. solanacea* were subjected to preliminary phytochemical analysis for the detection of phytochemicals such as alkaloids with Mayer's and Hager's reagent, Carbohydrates with Benedict's test and Fehling's test, glycosides with Legal's test and Modified Borntrager's test, phytosterols with Salkowski's test and Libermann Burchard's test, proteins with xanthoproteic test, flavonoids with alkaline reagent test and lead acetate test, tannins with gelatin test, saponins with Froth test and foam test, phenols with ferric chloride test[14].

Antioxidant activity

Table 6: Summary of the cytotoxicity in Brine Shrimp bioassay

Serial No	Sample Name	Regression Line	R^2	LC_{50} value
1	Vincristine (Positive Control)	y = 33.22x + 58.78	R² = 0.958	0.544
2	*Ardisia solanacea* Methanolic extract	y = 27.58x + 42.59	R² = 0.860	1.856
3	*Ardisia solanacea* Petroleum ether fraction	y = 29.39x + 29.33	R² = 0.944	0.7033
4	*Ardisia solanacea* Carbon Tetra Chloride fraction	y = 14.29x + 67.16	R² = 0.925	0.0629
5	*Ardisia solanacea* Chloroform fraction	y = 30.60x + 21.82	R² = 0.972	8.33
6	*Ardisia solanacea* aqueous fraction	y = 18.52x + 58.89	R² = 0.929	0.3311

There are various well known methods, which are followed to determine the antioxidant properties. Among them, two complementary test methods namely total phenolic content determination and DPPH free radical scavenging assay methods were used for investigating the antioxidant activity of *A. solanacea*.

Table 7: Time taken for paralysis and death of earthworms for methanol extract of *A. solanacea*, standard drug and control

Test substance	Conc. (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control (Distilled water)	0	-	-
Standard (Piperazine)	10	14.74 ± 0.408	23 ± 1.433
Methanol extract of *A. solanacea*	10	91.25± 1.796	101± 2.396
	20	67.25 ± 2.495	72.5 ± 2.658
	40	59.5 ± 3.175	67.75 ± 2.720
	60	46.5 ± 2.062	54.25 ± 1.548
	80	44 ± 1.826	49.5 ± 1.848

Total phenolic content determination

The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent[4]. Here, gallic acid was used as a standard and the amount of total phenolics were expressed as mg/g of gallic acid equivalents (GAE). Concentration of 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 μg/ml of gallic acid and concentration of 2 μg/ml of plant extract were prepared in methanol and 0.5 ml of each sample were placed into test tubes and mixed carefully with 2.5 ml of a 10- fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The test tubes were covered with para-film and allowed to stand for almost 30 min at room temperature before the absorbance was read at 760 nm spectrophotometrically (UV-1800, Shimadzu, Japan). All determinations were performed nicely in triplicate[4]. Thus, total phenolic content was determined as mg of gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve.

DPPH scavenging activity

DPPH radical serves as the oxidizing radical to be reduced by the antioxidant (AH) and as the indicator for the reaction. The stable DPPH radical-scavenging activity was measured using the modified method described by Gupta[15]. In this assay, 2 ml of 0.2 mμ methanolic DPPH solutions was added to 2 ml of extract solution at different concentrations and the contents were stirred vigorously for 15 seconds. Then the solutions were allowed to stand at dark place at room temperature for 30 min for reaction to occur. After 30 min, absorbance was measured against a blank at 517 nm with a double beam UV/Visible spectrophotometer. The percentage of DPPH radical-scavenging activity of each plant extract was calculated as DPPH radical-scavenging activity (%I),

$$= \frac{A_0 - A}{A_0} \times 100$$

Where,

A0 is the absorbance of the control solution (containing all reagents except plant extracts);

A is the absorbance of the DPPH solution containing plant extract.

The DPPH radical-scavenging activity (%) was plotted against the plant extract concentration to determine the concentration of extract necessary to decrease DPPH radical-scavenging by 50% (called IC50). The IC50 value of each extract was estimated by sigmoid non-linear regression. These values were changed to antiradical activity, defined as 1/EC50, since this parameter increases with antioxidant activity. All determinations were performed in triplicate.

Antimicrobial activity

Test Organisms

Two strains of Gram-positive (Staphylococcus aureus, Bacillus subtilis), three strains of Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi), and two strains of fungi (Candida albicans, Aspergillus niger) were used to evaluate the antimicrobial activity. The organisms were subcultured properly in nutrient broth and nutrient agar. They were collected from the, Chittagong Veterinary and Animal Sciences University (Department of Microbiology), Bangladesh.

For the evaluation of antimicrobial activity, disc diffusion method is widely acceptable[3]. In this classical method, antibiotics were diffused from a reliable source through the nutrient agar and a concentration gradient was created. Dried, sterilized filter paper discs (6 mm diameter, HI-Media, China) containing the test samples of known amounts (400 μg/disc) were placed on nutrient agar medium consistently seeded with the test microorganisms. Standard antibiotic of ciprofloxacin (5 μg/disc) and blank discs were used as positive and negative control. For the maximum diffusion of the test materials to the surrounding media, these plates were kept at low temperature (4 °C) for 24 h**.** Then the plates were incubated at 37 °C for about 24 h to allow optimum growth of the organisms. The test materials with antimicrobial property inhibited microbial growth in plates and thereby yielded a clear, distinct zone defined as zone of inhibition. The antimicrobial activity of the test sample was then determined nicely by measuring the zone of inhibition expressed in millimeter[3].

Thrombolytic activity

In vitroclot lysis activity of *A. solanacea* was carried out according to the method of Prasad et al., 2007[16] with minor modifications

Streptokinase (SK)

To the commercially available lyophilized S-Kinase™ (Streptokinase) vial (Batch no: VEH 09, Popular Pharmaceuticals Ltd., Bangladesh) of 15, 00,000 I.U., 5 ml 0.9% sodium chloride (NaCl) was added and mixed properly. Then the solution was diluted up to 300000IU and 15000IU conc. which was used as the reference standard for thrombolytic activity[16].

Specimen
Venous blood (5 ml) was drawn from healthy human volunteers (n =10) without a history of oral contraceptive or anticoagulant therapy (using a protocol approved by Institutional Ethics Committee). A consent form was filled up for every volunteer before collecting the blood .500 µl of blood was transferred to each of the previously weighed micro centrifuge tubes to form clots[16]

Preparation of sample
The prepared 0.9% NaCl solution was used to make different concentrations of plant extract: 5, 10 and 20 mg/mL.

Study design
Venous blood drawn from healthy volunteers (n = 10) was immediately citrated using 3.1% sodium citrate solution and then was transferred in different pre-weighed sterile micro centrifuge tube (500 µl/tube). Two hundred microlitre of 2% calcium chloride was then added to each of these tubes, mixed well and incubated carefully at 37°C for 45 minutes for clotting to occur. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed consciously to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). Each micro centrifuge tube containing clot was properly labeled and five hundred microlitre of different concentrations of the plant extract such as 5 mg/mL(n = 10), 10 mg/mL (n = 10) and 20 mg/mL (n = 10) or saline (negative control) (n =10) or 30,000 I.U. or 15000IU reference drug (n = 10)] was added to tubes with clots. All the tubes were incubated at 37ºC for almost 90 min. The fluid left was then carefully removed and the tubes were weighed again properly. The difference in weight before and after clot lysis was expressed as % clot lysis[16]. The result was expressed as percentage of clot lysis following the underneath equation.

% of clot lysis = (wt. of released clot /clot wt.) × 100

Anthelmintic activity
The anthelmintic study was carried out by the method of Ajaiyeoba *et al.*[17] with minor modifications. Adult earthworms were used to study the anthelmintic activity because they are anatomically and physiologically resemble with the intestinal roundworm parasites of human being[2]. They are widely used as effective tools for anthelmintic study because of their easy availability[2]. The earthworms belonging to species *pheritima posthuma* (annelida), about 3-5 cm in length and 0.1- 0.2 cm in width weighing about 0.8-3.04 g, were collected from the moist soil of Noakhali Science and Technology University, Sonapur, Noakhali and identified by the Department of Fisheries and Marine Science (FIMS), Noakhali Science and Technology University (Voucher No. 112/2013). The crude methanolic extracts of *A. solanacea* were used as test samples. They were used to prepare different concentrations (10-80 mg/ml) separately. For the methanol extract different concentrations were prepared by weighing 100 mg, 200 mg, 400 mg, 600 mg and 800 mg extracts and dissolving them in 10 ml distilled water separately. 100 mg of piperazine citrate was measured by weighing machine and dissolved in 10 ml water to make a concentration of 10 mg/ml. A control group was established with distilled water to ensure that the test was a validate one. Earthworms were divided into twelve groups (each containing four earthworms) in petridish. Five groups were used for the five concentrations of methanolic extracts of *A. solanacea*. One group was applied to reference standard and another to control group. Finally, the time of paralysis and death was determined consciously. Time for paralysis was noted when no movement of any sort could be observed except when the worms were vigorously shaken. Time for death of worms was taken after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (almost 50 °C) followed with fading away of their body colors[2]

Cytotoxic activity
The cytotoxic property of the extract was determined using brine shrimp lethality test[2]. The investigation was done on *artemia salina* (brine shrimp). *Artemis Salina leach (*brine shrimp eggs) collected from pet shops was used as the test organism. One spoon of cyst was hatched for about 48 hrs in saline water, prepared by dissolving 20 g pure NaCl and 18 g normal edible NaCl into 1 L water. The hatched cyst in turn became living nauplii. Different concentrations of the extract were prepared using dimethyl sulfoxide (DMSO) as solvent. For the test, different concentrations of plant extract prepared were added to test tubes, each containing 10 shrimps in saline water. Here, vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get a primary concentration of 40µg/ml from which serial dilutions were made using DMSO to get 20µg/ml, 10µg/ml, 5µg/ml, 2.5µg/ml, 1.25µg/ml, 0.625µg/ml, 0.3125 µg/ml, 0.15625µg/ml and 0.78125µg/ml solution from the extract. Then the positive control solutions were added to the pre-marked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups.

Counting of Nauplii
After 24 hours, the vials were inspected by using a magnifying glass and the number of survived nauplii in each vial was counted consciously. From this result, the percent (%) of lethality of the brine shrimp nauplii was calculated nicely for each concentration.

Statistical Analysis
The results are expressed as mean ± SEM. Statistical comparisons were made using one-way ANOVA with Dunnett t test. Significance was set at $p < 0.05$. Dose dependencies were determined by the regression coefficient (r).

RESULTS

Phytochemical screening
The phytochemical screening of methanol extracts of *A. solanacea* showed varied results. The results are shown in Table 1.

Total phenolic content determination
The methanolic crude extract of ***A. solanacea*** and its different soluble fractions i.e. Petroleum ether, chloroform and carbon tetra chloride were subjected to total phenolic content determination. Based on the absorbance values of

the extract solution, the colorimetric analysis of the total phenolic of the extracts were determined and compared with the standard solutions of gallic acid equivalents (Table 2). Total phenolic content of the samples are expressed as mg of gallic acid equivalent (GAE)/ gm of dry extract. ***A. solanacea*** was found as a good source of total phenolic contents where crude methanolic, petroleum ether, carbon tetra chloride and chloroform extract showed total phenolic contents of 58.35µg of GAE / mg,69.41µg of GAE / mg, 37.41µg of GAE / mg and 10.82µg of GAE / mg extracts respectively.

DPPH scavenging activity

DPPH free radical scavenging activity of crude extracts of ***A. solanacea*** *and* their different soluble fractions were found to be increased with the increase of concentration of the extract (Table 3). Different partitions of methanolic extract of ***A. solanacea*** were subjected to free radical scavenging activity. Here, Ascorbic acid was used as reference standard. In this investigation, the petroleum ether soluble fraction showed the highest free radical scavenging activity with IC50 value 40.04 µg/ml. At the same time the methanolic crude extract, Carbon tetrachloride, aqueous and chloroform soluble fractions also exhibited moderate antioxidant potential having IC50 value 52.11 µg/ml, 91.34 µg/ml, 174.13 µg/ml and 157.39µg/ml respectively.

Antimicrobial activity

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms (at concentrations of 400 µg/disc.) surrounding the discs which gives clear zone of inhibition. After incubation, the Antimicrobial activities of the test materials such as Methanolic crude extract of ***A. solanacea*** (MAS), Petroleum ether fraction of crude extract (PEAS), Chloroform fraction of crude extract (CAS), Carbon tetra Chloride fraction of crude extract (CTCAS) and Aqueous fraction of crude extract (AAS) were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale (Table 4). Here, Standard antibiotic disc of ciprofloxacin (5 µg/disc) was used for the comparison.

The methanolic extract exhibited moderate activity against the growth of the most of the test organisms (Table 4), while other soluble fractions exhibited less than moderate inhibitory activity against the microorganisms and the chloroform extract exhibited poor inhibitory activity against the microorganisms (Table 4). The zones of inhibition produced by crude methanolic extract were ranged from 7-12.9 mm.

Thrombolytic potential

Streptokinase (30000 and 15000 I.U.) as a positive control to the clots along with 90 minutes of incubation at 37 degree centigrade, showed 45.35% and 26.14% clot lysis respectively. Clots when treated with 100 microliters 0.9% saline water (negative control) showed only 5.51% blood clot lysis. The mean difference in clot lysis percentage between positive and negative control was very significant. By comparing with the negative control the mean clot lysis percentages of methanolic and petroleum ether extract of ***A. solanacea*** was significant (p value <0.0009).

Anthelmintic test

The crude methanolic extracts of ***A. solanacea*** produced a significant anthelmintic activity in dose dependent manner and the activity of methanol extract was comparable with that of standard drugs, which is shown in table 6

Cytotoxic activity

The LC_{50} values of crude methanol extract, petroleum ether, chloroform, carbon tetra chloride and aqueous extract of ***A. solanacea*** found to be 1.856, 0.7033, 8.331, 0.062 and 0.331 µg/ml, respectively (Table 7). The positive control vincristine sulphate showed LC_{50} at a concentration of 0.544 µg/ml.

DISCUSSION

Recently, focus on plant research has increased throughout world[18]. Medicinal plants containing phytochemicals show a variety of pharmacological actions in human body[19] and in our study, phytochemical screening showed the presence of different phytochemicals. The presence of polyphenolic compounds such as flavonoids, phenols and tannins are responsible for antioxidant activity of the plant extracts[20]. For maintaining a healthy biological system, the balance between antioxidation and oxidation is believed to be critical[21]. In our investigation the plant extract showed moderate antioxidant activities with an IC50 which were compared with the values of standard drugs used. Antioxidant activities of different extracts of the bark of *A. solanacea* found to increase with the increasing concentration. Hence, the extracts of this plant could be used for the prevention of free radical-mediated diseases[3]. Since the present study showed the presence of different bioactive secondary metabolites such as tannins, flavonoids, saponin and alkaloids, that singly or in combination may be responsible to treat microorganisms and insects. For this reason, the plant extract contains antimicrobial activity.

Platelets play a significant role in the process of formation of thrombus on the endothelial surface[22]. Many thrombolytic agents are applied to dissolve the clots that have already formed in the blood vessels but these drugs have few limitations which can lead to serious fatal consequences[23]. In this study, the extracts of plant showed significant thrombolytic activity compared to negative control. This thrombolytic activity may be due to the fact that the extracts are rich sources of alkaloids, flavonoids, tannins and terpenoids which are said to exert clot lysis[24,25]

The extracts of *A. solanacea* leaves showed a significant anthelmintic activity in dose dependent manner. Phyto-constituents like alkaloids, tannins, phenols etc. may be responsible for anthelmintic property[26]. Alkaloids were reported to cause paralysis of the worms by acting on its central nervous system. On the other hand, The main effect of anthelmintic drug is to cause a flaccid paralysis of the worm[26]. This plant also revealed good cytotoxic potency. It is believed that plant extracts containing a higher concentration of bioactive compounds show cytotoxic activity. Flavonoids show anti-allergic, anti-inflammatory, antimicrobial, and anticancer activities. Few studies tend to suggest that tannins may contain significant cytotoxic and antitumor potency[4].

CONCLUSION

In the context of the above discussion, it can be revealed that the extracts of bark of *A. solanacea* showed moderate antioxidant activity. These extracts also possess significant antimicrobial, thrombolytic, anthelmintic and cytotoxic activity. However, further investigations, based on these preliminary researches are required to explore the bioactive molecules which are responsible for the extracts' activities as well as their mechanisms of action.

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