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Biological Investigations of Dried Fruit of *Solanum nigrum* Linn.

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ABSTRACT

The ethanolic extract of the dried fruit of *Solanum nigrum* Linn. (Family: Solanaceae) was assessed for its possible analgesic, antidiarrhoeal, antimicrobial, antioxidant and cytotoxic activity. Phytochemical screening of the ethanolic extract revealed the presence of carbohydrate, alkaloids, tannins, saponins, steroids, glycosides, and gums. In acetic acid induced writhing in mice, the ethanolic extract (250 and 500mg/kg) exhibited significant ($p<0.05$ & $p<0.01$) inhibition of writhing reflex 51.39% and 66.67% respectively compared to standard diclofenac sodium. The fruit extract showed a significant ($P<0.01$ and $P<0.001$) antidiarrhoeal activity against castor oil induce diarrhoea in mice in which it decreased the frequency of defecation and increased the mean latent period at the dose of 250mg/kg and 500mg/kg body weight. The ethanolic extract showed moderate antibacterial activity against both gram-positive and gram-negative bacteria. In the qualitative antioxidant assay using DPPH (1, 1-diphenyl-2-picryl hydrazyl) the extract showed free radical scavenging properties. In the brine shrimp lethality test, the extract showed cytotoxicity significantly with LC_{50}= 63.10µg/ml and LC_{90}= 160µg/ml. All the results tend to justify the traditional uses of the plant and require further investigation to identify the chemicals responsible for these effects.

Key words: *Solanum nigrum* Linn, analgesic activity, antidiarrheal activity, antimicrobial activity, antioxidant activity, cytotoxic activity.

INTRODUCTION

Solanum nigrum Linn. (Family: Solanaceae) is commonly known as tit begun, phuti begun, gurki, gurkamai and kakmachi. It is an annual herb, low growing soft plant with ovate or ovate-lanceolate leaves, small flowers in intra-axillary sub-umbellate, 3 to 8-flowered cymes and small globose, purplish black, smooth shining fruits in bunches, grows as a common weed in all areas of the country (Ghani, 2003).

Leaf is a rich source of riboflavin. It also contains nicotinic acid and vitamin C, beta-carotene, citric acid, protein, fat, steroidal glycol-alkaloids, solasonine and solamargine. Fruits contain saponins and the steroidal glycol-alkaloids, solanine, solamargine, solasonine, α and β-solanigrine and the aglycone, solasodine, steroidal genin, trigogenin. Seeds contain solanine, protein, greenish-yellow oil consisting linoleic, oleic, palmitic, and stearic acids and sitosterol (Ghani, 2003).

Fruits are used as tonic, diuretic and in the treatment of fever and diarrhoea. They are also used in the treatment of eye diseases, heart diseases, anasarca and hydrophobia. Juice of plant possesses sedative, hydragogue, diaphoretic, diuretic, alternative properties and is given in chronic enlargement of the liver, blood-spitting, piles and dysentery. Leaves and young stems have also similar properties. The plant is a remedy for anthrax pustules and is beneficial against skin diseases. Fifty percent alcoholic extract of the plant at the dose of 50 mg/100 g P.O. significantly lowered the lipid level and prevented development of fatty liver in albino rats. The juice of fresh leaves is reported to produce dilation of the pupil (Ghani, 2003).

MATERIALS AND METHODS

Sample collection and extraction

The fruits of *Solanum nigrum* Linn. (Family: Solanaceae) were collected from the road side area of Dumuria Thana, Khulna, Bangladesh in May, 2009 and identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. DACB- 34408). A voucher specimen has been deposited in Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh. The identified fruits were dried under shade. After complete drying, the sample was cut into small pieces and then slashed to coarse powder with the help of mechanical grinder and the powder was stored in a suitable container. About 500 mg of powder was extracted by maceration over 20 days with 1200ml of 80% ethanol. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through filter paper. The filtrate thus obtained was evaporated by using a rotary evaporator to get a viscous mass. The viscous mass was then vacuum-dried to get a dried ethanolic extract (approx. yield value 10%). The extract thus obtained was used for experimental purposes.

Animals

Swiss-Albino mice of either sex (20-25 gm body weight) were collected from animal resources branch of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and were used for the experiments. The animal were kept in the standard polypropylene cages and provided with standard diets (ICDDR, B formulated). The animals were acclimatized in animal house, Pharmacy Discipline, Khulna University, Khulna under standard Laboratory conditions (relative humidity 55-60%, room temperature 25±2℃ and 12 hours light: dark cycle) for period of 14 days prior to performing the experiments.

Microorganisms

Both gram positive and gram negative bacterial strain were collected from the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B).

Drugs and Reagents with source

The standard drugs Diclofenac sodium, Loperamide and Chloramphenicol were collected from Beximco Pharmaceuticals Ltd. Dhaka, Bangladesh.

Phytochemical tests

The crude extract was subjected to preliminary phytochemical screening for the detection of major functional groups (Evans, 1989). Then, the extract was used for pharmacological screening.

Determination of analgesic activity

The analgesic activity of the sample was studied using acetic acid induced writhing model in mice. Experimental animals were randomly selected and divided into four groups denoted as Control group, Positive control group and Test group I and Test group II consisting of five (05) mice in each group. Control group received orally 1% Tween-80 at the dose of 10mg/kg body weight and Positive control group received orally diclofenac sodium at the dose of 25mg/kg body weight. Test group I and Test group II were treated with test sample orally at the dose of 250 and 500gm/kg body weight. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intra-peritoneally to each of the animals of a group. After an interval of 5 minutes was given for absorption of acetic acid and number writhing was counted for 15 minutes. The animals do not always perform full writhing. The incomplete writhing was taken as half-writhing, so two half-writhing were taken as one full writhing. This is why total writhing was halved to convert all writhing to full writhing or real writhing (Whittle, 1964, Ahmed et al*.,* 2004).

Antidiarrhoeal activity test

Antidiarrhoeal activity of the ethanolic extract of the fruit of *Solanum nigrum* Linn. was tested using the model of castor oil induced diarrhoea in mice (Chatterjee, 1993). All the mice were screened initially by giving 0.5ml of castor oil and only those showing diarrhoea were selected for the experiment. The test animals were randomly chosen and divided into four groups having five (05) mice in each group. Group I was kept as “control” and received 1% Tween-80 at the dose of 10mg/kg body weight; Group II was “positive control” and received standard antimotility drug, Loperamide at the dose of 50mg/kg body weight as oral suspension; and Group III Group IV were “test group” and treated with suspension of fruit extract of *Solanum nigrum* Linn. at the oral dose of 250 and 500mg/kg body weight. Control vehicle and the extract were administered orally, 30min.

prior oral administration of castor oil at the dose of 0.5ml. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hours study after the castor oil administration. Number of stools on any fluid material that stained the adsorbent paper were counted at each successive hour during the experiment (05 hrs). The latent period of each mouse was also counted. At the beginning of each hour new papers were placed for old ones.

Antibacterial activity

Antibacterial activity of dried fruit of *Solanum nigrum* Linn. was tested by using the disc diffusion method (Bauer et al., 1966; Ahmed et al., 2003). Sample impregnated discs, standard antibiotic discs (Kanamycin) and negative control discs were placed gently on the seeded agar plates with the help of sterile forceps to assure complete contact with medium surface. The plates were then inverted and kept in refrigeration for about 2hr at 4℃ to allow the material to diffuse into a considerable area of the medium. Finally the plates were incubated upside down at 37℃ for 24h. After proper incubation, the antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition in terms of millimeter with a slide calipers.

Determination of Antioxidant Activity

Antioxidant activity was determined on the basis of their scavenging activity of the stable DPPH free radical (Sadhu et al., 2003). Commercially prepared TLC plate was used. The sample and ascorbic acid were spotted. Here ascorbic acid was used as standard. The chromatogram was developed by ascending technique using two types of solvent systems i.e. medium polar solvent system ($CHCl_3$: CH_3OH = 5:1) and polar solvent system ($CHCl_3$: CH_3OH: H_2O = 40:10:1). The solvent system was allowed to move upto a previously marked line. The plates were then dried naturally. The plates were viewed under UV detector both in short (254 nm) and long (360 nm) wavelength. DPPH (1, 1-diphenyl-2-picryl hydrazyl) forms deep pink color when it dissolved in ethanol. When it is sprayed on the chromatogram of the extract, it forms pale yellow or yellow color which indicates the presence of antioxidants. Two spotted TLC plates were again subjected to universal spry reagent i.e. 10% H_2SO_4 and then heated on hot plate which indicates dark spot.

Determination of Cytotoxic Activity

The brine shrimp eggs were hatched in a conical flask containing brine shrimp medium (300ml). The flask were well aerated with the aid of an air pump, and kept in a water bath at 29-30℃. A bright light was left on it. The nauplii hatched within 48 h. The extract was dissolved in brine shrimp medium with addition of few drops of 5% dimethyl sulfoxide (DMSO) to obtain a concentration of 5, 10, 20, 40, 80 and 160µg/ml. Each preparation was dispensed into clean test tubes in 10ml volumes and tested in duplicates. For control, same procedure was followed except test samples. A series of same concentration as of sample was prepared for positive control, chloramphenicol. After making the test tube properly, 10 living shrimps were added to each of the test tubes with the help of a Pasteur pipette. The test tubes containing the sample, control and positive control were then incubated at 29℃ for 24hr in a water bath, after which each test tube was examined and the surviving brine shrimp counted and recorded. From this, the percentage of mortality was calculated at each concentration to determine the LC_{50} (Meyer *et al.*, 1982).

Statistical analysis

Student's t-test was used to determine significant differences between the control group and test group.

RESULTS

In the preliminary phytochemical screening the extract showed the presence of reducing sugars, tannins, glycosides, alkaloids, saponins, gum, and steroids (Table 1).

Table 1: Results of preliminary phytochemical analysis

Plant Extract	Alkaloids	Glycosides	Steroids	Gums	Tannins	Saponins	Flavonoids	Reducing sugar
Ethanolic extract of *Solanum nigrum*	+	+	+	+	+	+	-	+

+ = Presence; - = Absence

Analgesic activity test

Analgesic activity of the ethanolic extract of *Solanum nigrum* Linn. fruit was tested by acetic acid induced writhing model in mice. The extract produced 69.45% ($p<0.01$) acetic acid induced writhing inhibition in mice at the dose of 500mg/kg body weight, which is comparable to diclofenac sodium 66.67% ($p<0.01$) at the dose of 25mg/kg body weight (Table 2).

Table 2: Effect of dried fruits of *Solanum nigrum* Linn. on acetic acid induced writhing in mice.

Animal Group	Treatment	Writhing Count (%Writhing)	%Writhing Inhibition
Control (n=5)	1% tween-80 solution in water	14.4 ± 2.37 (100)	0
Positive Control (n=5)	Diclofenac sodium (25mg/kg)	4.4 ± 0.50* (30.55)	69.45
Test group I (n=5)	Et. Extract (250mg/kg)	7 ± 0.71** (48.61)	51.39
Test group II (n=5)	Et. Extract (500mg/kg)	4.8 ± 0.74*** (33.33)	66.67

Values are expressed as mean ± SEM, SEM=Standard error of Mean, n=No. of mice, Et. = Ethanolic, *: $P < 0.01$; **: $P < 0.05$; ***$P < 0.01$ vs. control

Antidiarrhoeal activity test

Antidiarrhoeal activity of the ethanolic extract of *Solanum nigrum* Linn. fruit was tested by castor oil induced diarrhoea in mice. The extract caused an increase in latent period (1.07 and 1.38h) *i.e.* delayed the onset of diarrhoeal episode at the dose of 250 and 500mg/kg body weight respectively as compared to the standard antidiarrhoeal agent Loperamide where the mean latent period was 1.97h (Table 3a). The extract also decreased the frequency of defecation at the dose of 250 and 500mg/kg of body weight respectively where the mean number of stool at the 1st, 2nd, 3rd, 4th, and 5th hour of study were 1.8, 2.7, 2.1, 2.7, 2.9 and 2.4, 1.6, 2.4, 2.6, 1.8 respectively which was comparable to the standard drug Loperamide where the mean number of stool at the 1st, 2nd, 3rd, 4th, and 5th hour of study were 4.1, 1.5, 1.0, 1.2, and 0.6 respectively (Table 3b).

Table 3a: Effect of dried fruits of *Solanum nigrum* Linn. on castor oil induced diarrhoea in mice (Latent period).

Animal Group/ Treatment	Dose (p.o)	Latent period (h)
Group I (Control): 1% Tween-80	10ml/kg	0.62±0.05
Group II (Positive Control): Loperamide	50mg/kg	1.97±0.08*
Group III (Test Group): Et. Extract	250mg/kg	1.07±.10**
Group IV (Test Group): Et. Extract	500mg/kg	1.38±0.07*

Values are expressed as mean ± SEM (n=5); *: $P<0.001$, **$P<0.01$ vs. control; p.o: per oral

Antibacterial activity test

Table 4 showed the results of antibacterial test. The antibacterial activity was assessed against a panel of 10 pathogenic bacterial strains (Both gram positive and gram negative) at the dose of 250 and 500µg/disc, and the result were compared with the activity of the positive control, kanamycin (30µg/disc). At 250µg/disc, the extract showed no activity. At 500µg/disc, the extract showed activity only against *Enterococcus faecalis* (6mm), *Streptococcus agalactiae* (10mm) and *Pseudomonas aeruginosa* (7mm).

Table 3b: Effect of dried fruits of *Solanum nigrum* Linn on castor oil induced diarrhoea in mice (Number of stools)

Animal Group/ Treatment	Dose (p.o)	Period of study (hr)	Total Number of Stool
Group I (Control): 1% Tween-80	10ml/kg	1	4.9±0.80
		2	5.5±0.31
		3	2.6±0.16
		4	3.2±0.62
		5	3.0±0.25
Group II (Positive Control): Loperamide	50mg/kg	1	4.1±0.26**
		2	1.5±0.52*
		3	1.0±0.12*
		4	1.2±0.32***
		5	0.6±0.21*
Group III (Test Group): Et. Extract	250mg/kg	1	1.8±0.36***
		2	2.7±0.42*
		3	2.1±0.23
		4	2.7±0.15
		5	2.9±0.26
Group IV (Test Group): Et. Extract	500mg/kg	1	2.4±0.29**
		2	1.6±0.21*
		3	2.4±0.25
		4	2.6±0.20
		5	1.8±0.24**

Values are expressed as mean ± SEM (n=5); *: $P<0.01$; **: $P<0.02$; ***$P<0.05$ vs. control, Et. = Ethanolic

Table 4: Antibacterial activity of ethanolic extract of the dried fruit of *Solanum nigrum* Linn.

Bacterial Strains	Type of Bacterial Strains	Diameter of Zone of Inhibition in mm			
		Blank	Kanamycin (µg/disc)	Extract (250µg/ disc)	Extract (500µg/ disc)
Shigella flexneri	Gram(-)	-	18	-	-
Enterococcus faecalis	Gram(+)	-	15	-	6
Streptococcus agalactiae	Gram(+)	-	20	-	10
Shigella sonnei	Gram(-)	-	27	-	-
Shigella boydii	Gram(-)	-	20	-	-
Streptococcus pyogenes	Gram(+)	-	26	-	-
Shigella dysenteriae	Gram(-)	-	20	-	-
Pseudomonas aeruginosa	Gram(-)	-	15	-	7
Staphylococcus saprophyticus	Gram(+)	-	10	-	-
Escherichia coli	Gram(-)	-	21	-	-

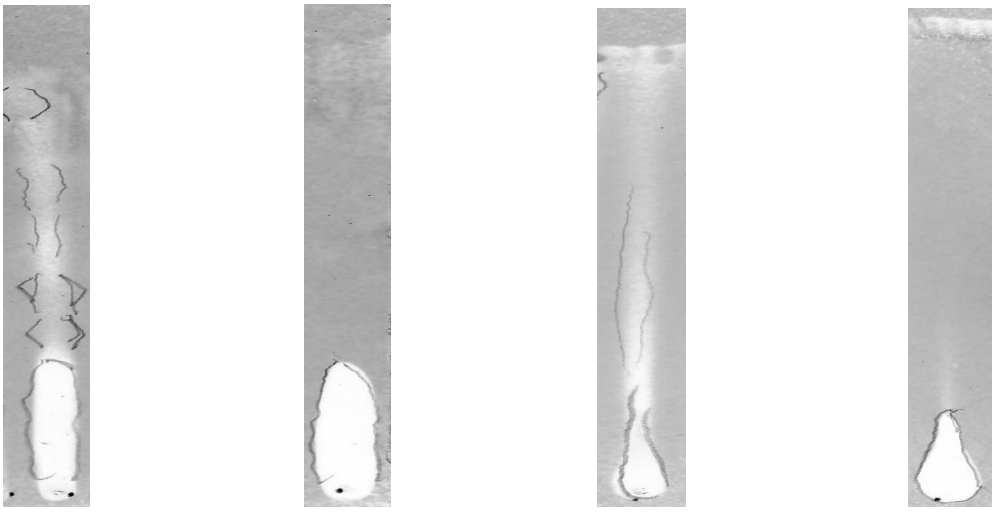
Gram (-):-Gram Negative Bacteria; Gram (+):-Gram Positive Bacteria; (-):- No inhibition

Antioxidant activity test

Only qualitative antioxidant activity was assayed using DPPH and ascorbic acid. Results are represented in the Figure 1a and Figure 1b for the medium polar and polar solvent system and showed antioxidant activity.

Cytotoxic activity test

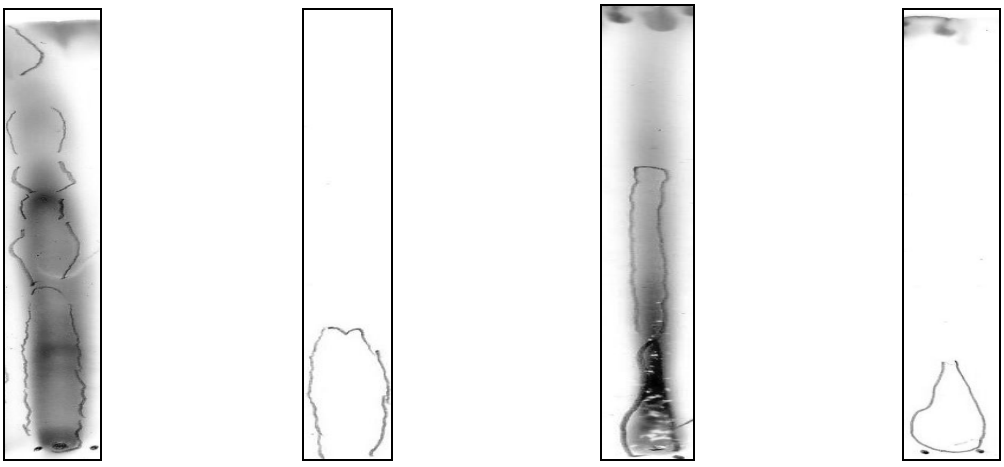
Brine shrimp lethality bioassay indicates cytotoxicity of extract. The extract was found to show lethal activity against brine shrimp nauplii and LC_{50} was found at 63.10µg/ml and the LC_{90} value was 160µg/ml respectively (Table 5a and Table 5b).



Sample **Standard (Ascorbic acid)** **Sample** **Standard (Ascorbic acid)**

(Chloroform: Methanol: = 5:1 as solvent) (Chloroform: Methanol: Water= 40:10:1 as solvent)

Figure 1a: Comparison of TLC plate for the dried fruit of *Solanum nigrum* with Standard (Ascorbic acid) after applying DPPH.



Sample **Standard (Ascorbic acid)** **Sample** **Standard (Ascorbic acid)**

(Chloroform: Methanol: = 5:1 as solvent) (Chloroform: Methanol: Water= 40:10:1 as solvent)

Figure 1b: Comparison of TLC plate for the dried fruit of *Solanum nigrum* with Standard (Ascorbic acid) after applying 10% H_2SO_4.

Table 5a: Result of Brine Shrimp lethality bioassay of ethanolic extract of the dried fruit of *Solanum nigrum* Linn.

Conc. (µg/ml)	Test-1	Test- 2	Avg. no of alive shrimp (sample)	Avg. no of alive shrimp (control)	% mortality	Log conc.
5	10	10	10		0	0.698
10	9	8	8.5		15	1.000
20	7	8	7.5		25	1.301
40	5	6	5.5	10	45	1.602
80	3	2	2.5		75	1.903
160	2	0	1		90	2.204
320	0	0	0		100	2.505

Table 5b: Result of Brine Shrimp lethality bioassay of Chloramphenicol

Group	Conc. (µgm/ml)	S-1	S- 2	Avg. no. of alive shrimp (sample)	Avg. no. of alive shrimp (control)	% mortality	Log conc.
Standard (Chloram-phenicol)	5	9	9	9	10	10	0.698
	10	8	8	8		20	1.000
	20	5	4	4.5		55	1.301
	40	4	3	3.5		65	1.602
	80	2	1	1.5		75	1.903
	160	0	1	0.5		95	2.204
	320	0	0	0		100	2.505

DISCUSSION

To get preliminary idea about the active constituents present in the fruit extract different chemical tests were performed and found the presence of Reducing sugar, Tannins, Saponins, Gums, Steroids, Alkaloid, Glycosides.

Analgesic activity of the ethanolic extract of *Solanum nigrum* Linn was tested by acetic acid induced writhing model in mice. Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings (Taesotikul et al*.,* 2003). Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Derardt *et al*., 1980). The ethanolic extract of *Solanum nigrum* Linn produced significant writhing inhibition comparable to the standard drug diclofenac sodium. On the basis of this result it can be concluded that the ethanol extract of *Solanum nigrum* Linn possesses analgesic activity.

Antidiarrhoeal activity of the ethanolic extract of *Solanum nigrum* Linn fruit was tested by castor oil induced diarrhoea in mice. Castor oil mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces it's anti-absorptive or anti secretory effect (Tripathi, 2001). The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that ricinoleate salts stimulate the intestinal epithelial cell's adenyl cyclase (Racusen et al., 1979) or release prostaglandins, which results in an increase in the net secretion of water and electrolytes in the small intestine (Beubler et al., 1979). The ethanolic extract of fruit of *Solanum nigrum* Linn significantly and dose dependently inhibited and delayed the onset of diarrhoea in mice. The maximum effect was found at 500mg/kg of body weight. On the basis of this result it can be concluded that the ethanol extract of *Solanum nigrum* Linn fruit might possess antidiarrhoeal activity.

Antibacterial activity was tested by using disc diffusion method. The extract showed activity only against *Enterococcus faecalis* (6mm), *Streptococcus agalactiae* (10mm) and *Pseudomonas aeruginosa* (7mm) among 10 species of bacteria. On the basis of this result it can be concluded that the ethanol extract of *Solanum nigrum* Linn fruit possesses moderate antibacterial activity.

Antioxidant activity of the ethanolic extract was determined on the basis of their scavenging activity of the stable DPPH free radical and 10% H_2SO_4. Only qualitative test was performed. Drugs with antioxidant activity are useful in free radical induced different types of diseases. The result might partially support the traditional uses of it for different tumors. Further studies as lipid per-oxidation inhibition, xanthin oxidase inhibition, erythrocyte membrane stability and other studies are essential to characterize them as biological antioxidants.

Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc. of the compound (Meyer et al., 1982; McLaughlin et al., 1988). The ethanolic extract of the fruit of *Solanum nigrum* Linn was found to show significant activity against the brine shrimp nauplii; LC_{50} was found 63.10µg/ml and LC_{90} was

found 160µg/ml. However, further investigations using carcinoma cell line are necessary to isolate the active compound(s) responsible for the activity.

CONCLUSION

According to above discussion *Solanum nigrum* Linn contains important chemical constituents that confer upon it as a medicinal agent. It was revealed that the fruit extract contains Reducing Sugar, Tannins, Saponins, Gums, Steroids, Alkaloids, and Glycosides which have potential role in its Analgesic, Antidiarrhoeal, Antimicrobial, Cytotoxic and Antioxidant activity. This could provide a rationale for traditional uses of this plant and suggests for further investigation and isolation of biologically active constituents responsible for the activity.

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