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PHYTOCHEMICAL INVESTIGATION, EVALUATION OF ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF THE METHANOL EXTRACT OF *CUSCUTA REFLEXA* (STEM)

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Abstract

Cuscuta reflexa is used for treating constipation, flatulence, body pains, itchy skin, frequent urination, dry eyes, ringing in the ears, lower back pain, blurred vision, tired eyes, hair growth promoters ability to prevent certain cancer cells and thus reduce the incidence of cancer. Its can build protection from cerebral ischemia, advance heart blood, tonify kidney. The present study was carried out to investigate phytochemical, antioxidant and cytotoxic activity of stem of *Cuscuta reflexa* methanolic extracts. The cytotoxic activity was determined by using brine shirmp lethality bioassay. For cytotoxic activity, extracts showed LC 50 183.64 µg/ml. On the other hand, the reference standard Vincristine sulphate showed LC 50 value was 7.27 µg/ml. It is also revealed that the extract of *Cuscuta reflexa* possess antioxidant activity IC50 157.80 µg/ml compare to standard IC50 64.38 µg/ml. The potential of these activities may be due to the presence of most of the phytochemicals which supports previous claims and validate its uses as an expected folk medicine.

Keywords: Cuscuta reflexa, Vincristine Sulphate, DPPH, Antioxidant

Introduction

Bangladesh is a land of numerous medicinal plants. About thousand out of estimated five thousands species in Bangladesh are regarded as having medicinal properties. In this study one of these plants, *Cuscuta reflexa* is Convolvulaceae family and is a group of 100- 170 species of yellow, orange, red or rarely green parasitic plants. Phytochemicals isolated from *Cuscuta reflexa* are flavonoids [1] dulcitol, mannitol, sitosterol, lycopene, apigenin-7-βrutinoside, quercetin, hyperoside [2-3] ,propenamide, reflexin, lutein, carotene, amarbellin, palmitic, oleic, stearic, linolenic acids, leuteolin, astragallin, benzopyrones, glucopyranosides, bergenin [4-6]. Lupeol isolated from *C. reflexa* is a pharmacologically active tri-terpenoids and posse's antimicrobial, anti inflammatory, antitumor, antiprotozoal and chemoprotective properties [7]. It also decrease interleukin 4 production by T-helper Type-2 cells [8-9]. Rural people of India used of *Cuscuta reflexa* for the treatment of jaundice, rheumatism and headache [10] and also used as urination disorders, muscle pain, cough, blood purifier. Seeds of *Cuscuta reflexa* have carminative and anthelmintic properties and used to treat bilious disorder [11]. *Cuscuta reflexa* is used in the treatment of constipation, flatulence, body pains, itchy skin, frequent urination, dry eyes, ringing in the ears, lower back pain, blurred vision, tired eyes,hair growth promoters ability to prevent certain cancer cells and thus reduce the incidence of cancer. Its can increase resistance to cerebral ischemia, enrich heart blood, tonify kidney [12].

Free radicals, which have one or more unpaired electrons, are produced in normal or pathological cell metabolism. Reactive oxygen species (ROS) can easily initiate the peroxidation of the membrane lipid. The peroxidation items independent from anyone else and their optional oxidation items, for example, malondialdehyde (MDA) and 4-hydroxinonenal can respond with organic substrates, for example, protein, amines and deoxyribonucleic acid [13]. Excessive generation of ROS induced by various stimuli and leads to a variety of pathophysiological processes such as inflammation, diabetes, hepatic damage, genotoxicity and cancer [14]. Antioxidant is used to control the special disease. Different photochemical parts particularly polyphenols, (for example, flavonoids, phenolic acids, tannins, and so on.) are known to be in charge of the free radical searching and cell reinforcement exercises of plants [15]. Shrimp lethality bioassay is a straightforward, high throughput cytotoxicity trial of bioactive synthetic substances. It is based on the killing ability of test compounds on a simple zoological organism-brine shrimp (*Artemia salina*). The shrimp lethality bioassay is broadly utilized in the assessment of poisonous quality of substantial metals, pesticides, medicines particularly regular plant separates and so on. It's a primer harmfulness screen for further tests on mammalian creature models. This bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, antiviral, pesticidal & anti-tumor etc. of the compounds [16].

Methods

Collection of plant material

For this present investigation the *Cuscuta reflexa* collected from the Babukhan, Rangpur, Bangladesh.

Preparation of the extract

The harvested parts of the plant were separated from undesirable materials. They were air dried for several weeks after cutting into small pieces. The parts of the plant were ground into a coarse powder with the help of a suitable grinder. The powder was put away in a airtight sealed compartment and kept in a cool, dim and dry spot. Then, the plant powder will dissolve in methanol solution in a flat bottom sealed container for several days. Then metabolic extract were evaporates by the rotary evaporator and plant extract were collect.

Phytochemical screening

Various phytochemical tests which were performed under the heading of phytochemical screening are general tests for Alkaloids by Mayer's Test, Hager's test, Dragendroff's Test, test for Carbohydrates by Molish test, Benedict test, Fehling's Test, test for Tannins by $FeCl_3$, test for Saponins by Froth test, test for Phenols by $FeCl_3$, test for Flavonoids by Alkaline, Lead acetate, test for Steroid by Salkowski Tests, Lieberman Burchardt test, test for Protein and amino acid by Xanthoproteic Test, and test for Diterpenens by Cu acetate [17-18]].

Antioxidant test

DPPH radical scavenging assay

Stock solution of the plant extract was prepared in ethanol from which a serial dilution was carried out to obtain concentration of 1, 5, 10, 50, 100, 500 µg/ml. Diluted solutions (2ml) were added to 3 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 minutes for reaction to occur.

Preparation 0.004% DPPH solution

4mg of DPPH was measured and dissolved in 100ml of ethanol thus 0.004% DPPH solution was prepared.

Procedures

At first 6 volumetric flasks are taken to make 6 unique kinds of concentration (1, 5, 10, 50, 100 and 500 µg/ml). Test tubes and volumetric flasks are rapped with foil paper. In 6 volumetric flaks serial dilution of extract is done and marked them respectively.1ml of sample from each concentration and 3 ml of 0.004% DPPH solution is taken with the help of pipette in 6 test tubes correspondingly. Then solution is kept in dark place for 30 minutes with raping each test tube with foil paper. In another test tube 3ml 0.004% DPPH & 1ml methanol is taken to prepare blank solution. Then absorbance is taken by UV Spectroscopy [19-21]. The percent of inhibition is calculated by using following formula

%inhibition= {(Blank absorbance- solution)/Blank absorbance}*100

Preparation of TBA Reagent

The standard solution of 4.0 mM of TBA was set up in glacial acidic acid. For this reason, 57.66 mg of TBA was disintegrated in 100 mL of glacial acidic acid.

Preparation of MDA and Calibration Standards

Standard stock solution of MDA (1 mM) was set up in glacial acetic acid. MDA (31.35 mg) was precisely gauged and dissolved in 100 mL solvent. From the stock solution, various groupings of 0.1, 0.2, 0.4, 0.6, and 0.8 mM were readied. The calibration curve was developed in the fixation scope of 0.1 to 1.0 mM.

Preparation of sample

TBA strategy, depicted is as per the following: The last sample concentration of 0.02% w/v was utilized in this technique. Two mL of 20% trichloroacetic acid and 2 mL of 0.67% of thiobarbituric acid were added to 1 mL of test solution. The blend was set in a bubbling water bath for 10 min and after that centrifuged subsequent to cooling at 3000 rpm for 20 min. The absorbance action of the supernatant was estimated at 552 nm and recorded after it has achieved its most extreme [22].

% Inhibition = 100 – [(A1 – A0) ×100]

Where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts.

Brine shrimp lethality test of crude extracts

Seawater was placed in a cleanser case (divided into dark and light area). Shrimp eggs were added to the dark side and secured. The set up was left in a sufficiently bright spot for 48 h. The hatched eggs, which swarm to the lit side, were utilized for the bioassay. 20 mg of every one of the concentrates was disintegrated in 5 ml of sea water. From this solution, 400, 200, 100, 50, 25, 12.5ml and that consistently decrease solution were moved into vials and made up to 5 ml. Ten (10) shrimps (nauplii) were moved into every one of these vials using Pasteur pipette. Repeats of every one of the portion levels were readied, utilizing seawater as control.

Number of survivors, passings, and nauplii with lazy development were recorded, 24 h later. Information were prepared (Using probit analysister) to evaluate LC50 values at 95% certainty interim for measurably noteworthy correlations of potencies; LC50 under 100 ppm was considered as potent [23].

Results

The crude methanolic extract of stems exhibit remarkable, the value is 157.80 that is higher than IC50value. Due to this antioxidant potential, further investigation is required to identify which metabolite is responsible to exert this effect. The results of antioxidant activity in *cuscuta reflexa* stem obtained by extraction methods were 8.12(% of inhibition). Whereas, the antioxidant activity of malondialdehyde (standard) were 96.22 (% of inhibition). The results of antioxidant activity in *cuscuta reflexa* stem are considerable. Vincristine sulphate are used as a standard positive control and the LC50 compared with negative control.

The LC50 of the crude extract and standard are 183.64 and 7.27 µg/ml; respectively.

Discussion

The advantageous impacts got from phenolic mixes have been credited to their antioxidant activity [24]. The data obtained from this study, DPPH radical scavenging activity of Ascorbic acid (IC50 =64.38 µg/ml) of Cuscuta reflexa was similar to the standard (IC50 =157.80µg/ml). TBA assay of malondialdehyde 96.22 and Methanolic extract of stem is 8.12. These discoveries concur with past reports on scavenging of free radicals [25]. The decreasing properties are for the most part connected with the presence of reductones, which have been appeared to exert antioxidant action by breaking the free radical chain by giving a hydrogen atom [26]. Polyphenolic compounds, similar to flavonoids, tannins and phenolic acids, usually found in plants have been accounted for to have different natural impacts, including antioxidant activity [27].

Braine shrimp lethality is a general bioassay, which is characteristic of cytotoxicity exercises, pesticidal impacts and different pharmacologic activities. The outcomes show the capacity of the plant concentrate to kill malignancy cells in cell cultures, kill pests, and apply a wide scope of pharmacologic impacts. The presence of saponins, alkaloids and cardiac glycosides may be responsible for the observed brine shrimps lethality activities of the extracts [28].

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Table 1: Qualitative chemical analysis of various extracts of *Cuscuta reflexa*

Phytochemical constituents	Methanolic extraction of stem
1.Alkaloids	
(Mayer's Test)	+
(Hager's test)	+
(Dragendroff's Test)	+
2.Carbohydrates	
(Molish test)	+
(Benedict test)	+
(Fehling's Test)	+
3.Tannins	
($FeCl_3$)	+
4.Saponins	
(Froth test)	+
5.Phenols	
($FeCl_3$)	+
6.Flavonoid	
(Alkaline)	+
(Lead acetate)	+
7.Protein and amino acid	
(Xanthoproteic Test)	-
8.Diterpenens	
(Cu acetate)	+
9.steroids	
(Salkowski Tests)	+
(Lieberman Burchardt test)	+

(+) = Indicates the presence of the tested group, (–) = Indicates the absence of the tested group.

Table 2: IC50 value of Ascorbic acid (Asc) and crude methanolic stem extract of *Cuscuta reflexa*

		Standard (Ascorbic acid)			Methanolic Extract		
Conc. (µg/ml)	**Abs. of Blank solution**	**Absorbance (nm)**	**% of Inhibition**	**IC_{50}**	**Absorbance (nm)**	**% of Inhibition**	**IC_{50}**
500	0.565	0.043	92.39	**64.38** µg/ml	0.057	89.91	**157.80** µg/ml
250		0.095	83.19		0.128	79.12	
125		0.145	74.34		0.142	74.87	
62.5		0.182	67.79		0.251	55.58	
31.25		0.219	61.24		0.356	36.99	
15.625		0.273	51.68		0.413	26.90	
7.813		0.308	45.49		0.435	23.00	
3.906		0.371	34.34		0.476	15.75	
1.953		0.417	26.19		0.498	11.86	
0.977		0.456	19.29		0.523	7.43	

Table 3: % of inhibition of stem extract of *Cuscuta reflexa* by TBA assay method

Name	% of inhibition
Malondialdehyde	96.22
Methanolic extract of stem	8.12

Table 4: Results of the test samples of *Cuscuta reflexa* for cytotoxic evaluation

Sample	LC_{50} (µg/ml)	Regression equation	R^2
Vincristin sulphate	7.2653	y = 6.515x + 2.666	0.985
Methanolic extract of *Cuscuta reflexa*	183.64	y = 0.232x + 7.396	0.916

Table 5: Effect of Vincristine Sulphate (positive control) on brine shrimp nauplii.

Test tube No.	Concentrtion (C) (µg/ml)	LogC	% Mortality	LC_{50} (µg/ml)
1	0.078	-1.108	1	7.2653
2	.156	-0.807	2	
3	.312	-0.506	3	
4	.625	-0.204	5	
5	1.25	0.097	15	
6	2.5	0.398	35	
7	5	0.699	50	
8	10	1	55	
9	20	1.301	60	
10	40	1.6020	65	

Table 6: Effect of crude methanolic extract of *Cuscuta reflexa* on brine shrimp nauplii

Test tube No.	Concentrtion (C) (µg/ml)	LogC	% Mortality	LC_{50} (µg/ml)
1	0.78	-0.1072	1	183.64
2	1.56	0.19382	2	
3	3.12	0.49485	3	
4	6.25	0.79588	5	
5	12.5	1.09691	8	
6	25	1.39794	15	
7	50	1.69897	21	
8	100	2	50	
9	200	2.30102	65	
10	400	2.60205	90	

Figure 1: Determination of antioxidant activity of stem extract of *Cuscuta reflexa* by TBA method

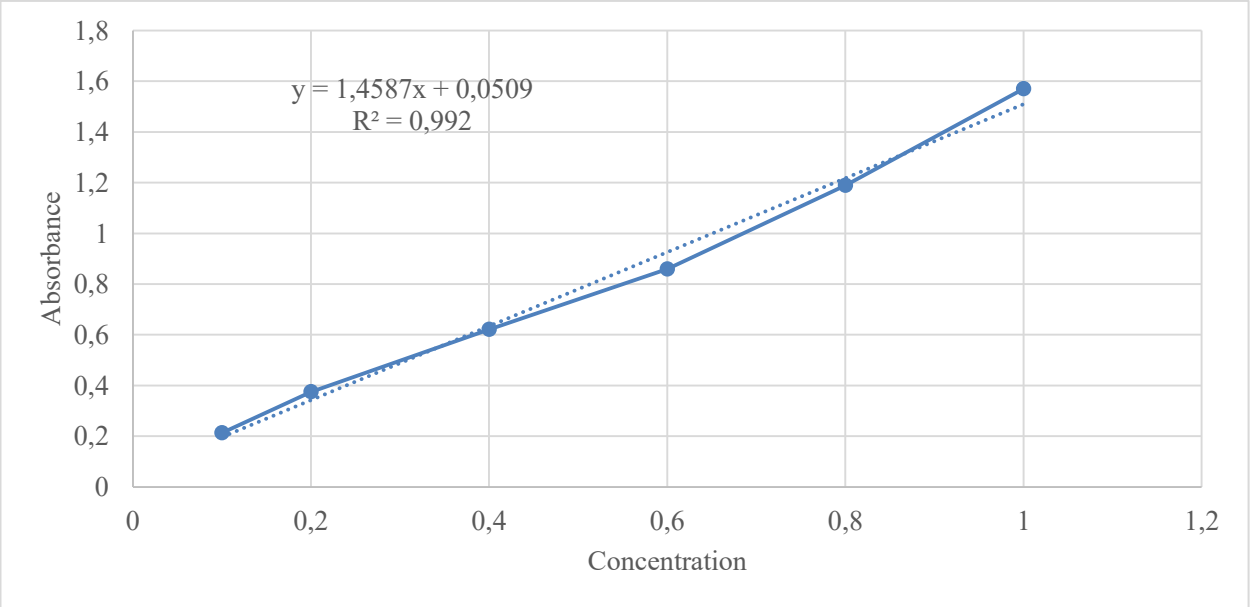

Figure 2: Effect of Vincristine Sulphate on Brine Shrimp Nauplii

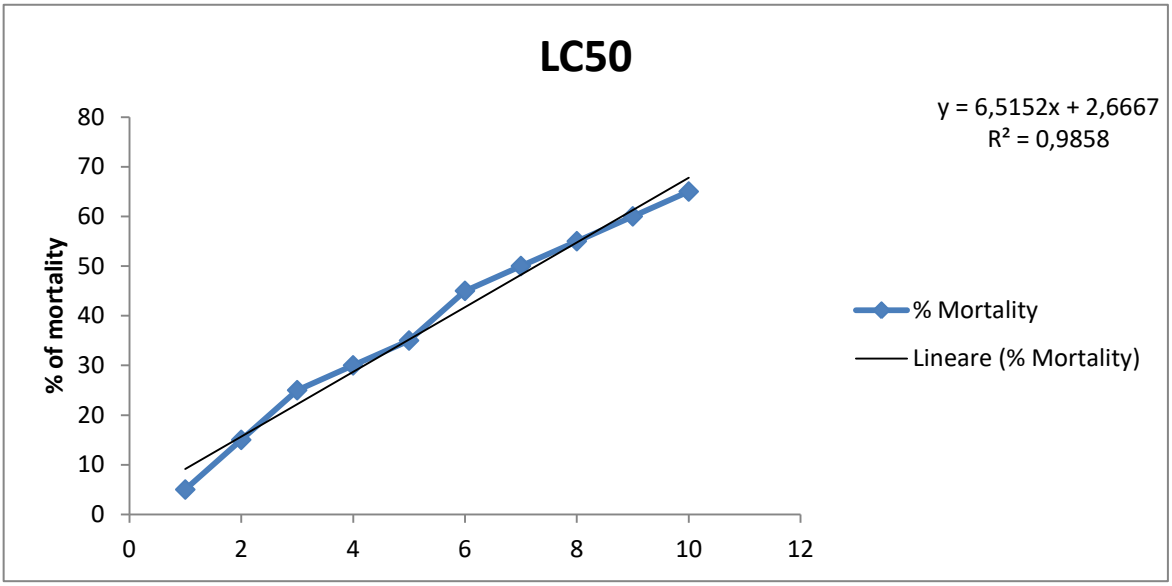

Figure 3: Effect of crude methanolic extract of *Cuscuta reflexa* on brine shrimp nauplii