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SCREENING OF IN VITRO ANTIOXIDANT, CYTOTOXIC AND ANTIMICROBIAL ACTIVITIES OF BELAMCANDA CHINENSIS AERIAL PART

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ABSTRACT

The utilization of medicinal plants to treat infectious disease is a common practice in developing countries worldwide. The present study was aimed at evaluating the crude extracts of Belamcanda chinensis (Aerial parts) with different chemicals for antioxidant and antimicrobial (fungal and bacterial) potential. All the partitionates were also subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this study, methanol and ethyl acetate soluble fractions were found to be highly toxic to Brine Shrimp nauplii, with LC50 of 16.218µg/ml and 0.048µg/ml respectively while the LC50 of the reference anticancer drug tamoxifen BP 20mg was 0.003µg/ml. All fractions produced concentration dependent increment in percent mortality of Brine Shrimp nauplii indicating the presence of cytotoxic principles in these extractives. Antimicrobial

activity of leaves of Belamcanda chinensis at a concentration of 0.01mg/10ml with solvent ethanol. Tectorigenin (present in very little content in aerial parts) was found to have strong antifungal activity against dermatophytes of the genera Trichophyton with MIC ranging from 3.12-6.25ml. These results also lend support to the relevant phytochemical and pharmacological works carried out so far on Belamcanda chinensis. However, further studies are suggested to be undertaken to understand the underlying mechanism of the observed activities and to isolate, purify and characterise active phytochemical ingredient(s) responsible for these bioactivities in animal models.

KEY WORD: Ethnobotany, cytotoxic, Belamcanda chinensis, Tectorigenin.

INTRODUCTION

Medicinal plants have been identified and used throughout human history. In developing countries including Bangladesh, about 75% of the populations rely on different forms of traditional medicine for their primary health care.[1] The high cost of imported conventional drugs and/ or inaccessibility to western health care facility, imply that traditional mode of health care is the main form of health care that is affordable and available to our rural people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective. As a result, traditional medicines usually exist side-by-side with western forms of medicine.

The use of plants as medicines predates written human history. Ethnobotany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant.[2]

Using Belamcandachinensis as medicinal plant

At present, numerous medicinal plants and related products are used for the treatment of various diseases. Chinese herbal medicines have been used for the treatment of human diseases for centuries. Belamcandachinensis(L.) DC is from the family of Iridaceae, which comprises approximately 60 genera and 800 species worldwide. However, only two genera (Iridacea and Belamcanda) are distributed in China. B. chinensis shrubs primarily grow in the southwest area of China, especially in the provinces of Guizhou, Yunnan, and Sichuan[3] and the species was listed as an official drug in the Chinese Pharmacopoeia (2005). The English

name of Belamcandachinensisis leopard lilyLeopard lily is also known as Blackberry lily. It's a rhizomatous erect perennial having a height of 2-3 ft. Leafs are sword shaped and flowers are loosely clusters. Color of the flower is orange and having dark red/brick colored spots on it. A centered stigma is surrounded by three stamens having yellow filaments. The seed of the plant is poisonous; also part of the plant is poisonous when ingested. The rhizome of B.chinensishas been used as a medicinal plant for over 2000 years, and it remains widely used in the treatment of several diseases, such as pulmonary disease, acute and chronic pharyngitis, asthma, and cancer In our recent work, the rhizomes of B. chinensiswere extracted using different solvents, and the antitumor activities against the PC3, Bcap-37, and BGC-823 cell lines, as well as the 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) free radical scavenging activity of the crude extracts, were tested. The results revealed that ethyl acetate extracts exhibited significant antitumor activities and moderate DPPH free radical scavenging effects.[4]

Introduction to Belamcanda chinensis

Taxonomic hierarchy of Belamcanda chinensis

Kingdom: Plantae

(Subkingdom): Tracheobionta– Vascular plants

(Superdivision): Spermatophyta – Seed plants

(Division): Magnoliophyta– Flowering plants

(Class): Liliopsida

(Subclass): Liliidae

Order: Asparagales

Family: Iridaceae

Genus: Iris

Species: I. domestica

Plant Description: General information

Belamcanda chinensis, the sole species in the genus Belamcanda, was transferred to the genus Iris and renamed Irisdomestica. Othersynonymsare Belamcandapunctata Moench, Gemmingiachinensis (L.)Kuntze, Irischinensis Curtis, Ixiachinensis L., Moreachinensis, an d Pardanthuschinensis (L.) Ker Gawl.)The plant grows 60–90 cm tall in full sun and is often found blanketing hillsides; the flowers can range from red to orange to yellow, or mixed, and bloom in summer to early autumn (fall). The leaves grow in a fan, like those of a gladiolus.

The flowers are typically orange spotted with red, although yellow- flowered varieties are in cultivation. The seed pods open in the fall, showing clusters of black seeds whose fancied resemblance to ablackberry gives the plant its common name, "blackberry lily". The plant is hardy to USDA plant hardiness zone 5 and is propagated by seeds or division.[5]

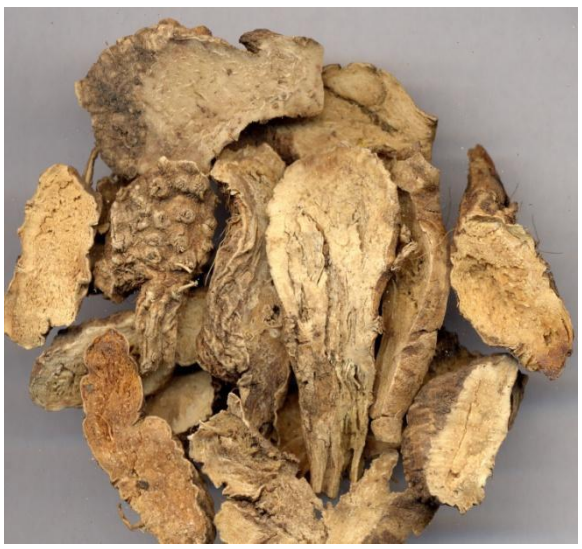
Common names

Blackberry lily, Leopard flower, Leopard lily, Chapta spider.

(A)

(B)

(C)



(D)

Figures: Different parts of Belamcanda chinensis. (a): Flower, (b): Seed, (C): Leaf (D): Rhizome.

Identification

Blackberry lily (Belamcanda chinensis) flower, about one and a half inches across, blooms for one day. The flower closes up, its tepals twist into a spiral shape, and the overy becomes the seed capsule. Blackberry lily plants continues flowering even as it develops seeds in the capsules. When mature, the seed capsule opens to reveal the shiny black seeds. Most of these seeds remain on the plant throughout the fall and winter. When spring arrives, the seeds drop to the ground.[6]

Habitat

Belamcanda chinensis is commonly found innative of central Asia, India, China and Japan has naturalized in the eastern and central regions of the United States.[7]

MATERIAL AND METHOOD

Collection and identification of Belamcanda chinensis

The whole plant was collected from F.H. Nursery, Banani, Dhaka in January 2014 during. The whole plant with leaves, flower, stems and roots was collected and identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka, where a Voucher specimen (Accession No. 39588) has been deposited for future reference.

Figure: National Herbarium sheet with accession number.

Drying of the whole plant of Belamcanda chinensis

The plant was thoroughly washed with water. Roots and the aerial parts were sliced into small pieces and drying by natural sunlight. The aerial parts and the roots parts dried separately. After 25 days of drying, it is prepared for grinding. The plant parts were dried in that at reduced temperature (at 550 C) to make them suitable for grinding process.

Grinding and storage of the dried samples

The dried aerial parts and root parts were ground to coarse powder with a mechanical grinder (Grinding Mill)seperately. This process breaks the plant parts to smaller pieces thus exposing internal tissues and cells to solvents thus facilitating their easy penetration into the cells to extract the constituents (Ghani, 2003). Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder. The weight of the total dry aerial parts powder was 200 g and root parts powder was 200g.[8]

Extraction of the dried powdered sample

The dried aerial parts and root parts of Belamcanda chinensis were coarsely powdered by a milling machine and extracted exhaustively with methanol (500ml for 50g) and with ethyl acetate (1000ml for 100g) in a Soxhlet apparatus for 72 hours for each attempt of extraction of the total powder. Aerial parts powder and root parts powder were socked separately.

After completion of the extraction, the liquid was filtered using a sterilised cotton filter. Then by rotary drying process the solvent was completely removed and obtained from methanol aerial parts 14.8052g and from ethyl acetate aerial parts 5.2689g[8] for rest of the experiments.

Figure: Rotary Evaporator.

Tests for antioxidant activity by DPPH radical scavenging assay

DPPH free radical scavenging activity of the plant fractions was determined following the method described by.[9]

Reagents and chemicals

Table: List of the reagents used in DPPH test and their sources.

Reagents used	Source
DPPH (1,1-diphenyl-2-picryl hydrazyl)	Sigma Chemicals, USA
Methanol	Merck, Germany
Ascorbic acid as standard	SD Fine chem. Ltd., Biosar, India

Principle

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can generate stable free radicals in aqueous or methanol solution. With this method it was possible to determine the antiradical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. Resulting from a colour change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form, this molecule has an absorbance at 517 nm which disappears after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.[8]

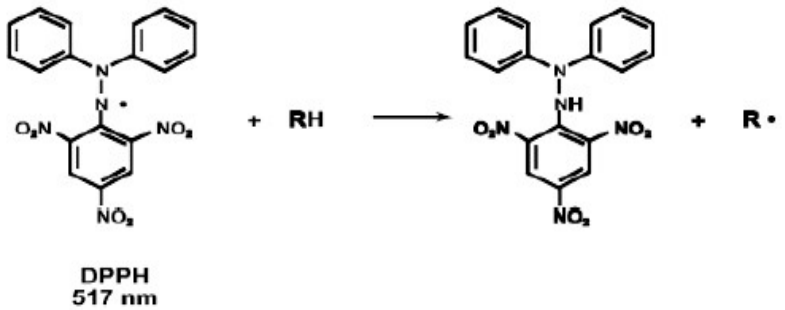


Figure: Structure of DPPH.

Experimental procedure

1. 10 ml of each fraction and standard (ascorbic acid) in different concentrations were taken in test tubes.
2. 2 ml of a 0.004% methanol solution of DPPH was added into the test tubes.

3. The test tubes were incubated for 30 minutes to complete reaction.
4. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer (Shimadzu UV PC-1600) against blank (methanol).
5. The percentage (%) scavenging activity was calculated from the following

Equation:

$$\% \text{ Scavenging} = \{(A0 - A1)/ A0\} \text{ X } 100$$

Where,

A0 is the absorbance of the control, and

A1 is the absorbance of the samples/standard.

6. Then % scavenging was plotted against concentration and IC50 was calculated.

Brine Shrimp lethality bioassay for cytotoxic activity

Cytotoxicity of the plant extractives was determined by Brine Shrimp lethality bioassay.

Principle

Brine Shrimp eggs are hatched in simulated seawater to get nauplii. Sample solutions are prepared by dissolving the test materials in pre-calculated amount of DMSO. Ten nauplii are taken in vials containing 5 ml of simulated seawater. The samples of different concentrations are added to the pre-marked vials with a micropipette. Survivors are counted after 24 hours. The median lethal concentration, LC50 values of the test samples after 24 hours are obtained by a plot of percentage of dead Shrimps against the logarithm of the sample concentration using Microsoft Excel. Vincristine sulphate is usually used as the reference cytotoxic drug.[9]

Materials

1) Artemia salina Leach (Brine Shrimp)

2) Sea salt (NaCl)

3) Small tank and test tubes.

4) Pipettes and Micropipette

5) Vincristine sulphate inj. and DMSO.

Table: Test samples of crude of Belamcanda chinensis for cytotoxic activity.

Test samples	Measured Amount (gm)
BCLM	0.004 gm
BCLE	0.004 gm

BCLM= Methanolic fraction of leaf, BCLE= Ethyl acetate fraction of leaf of Belancanda chinensis

Experimental procedure

Preparation of seawater

38 g sea salt (pure NaCl) was weighed, dissolved in 1 litre of distilled water to make preparation like sea water.

Hatching of Brine Shrimps

Artemia salina Leach (Brine Shrimp eggs) collected from Katabon (Dhaka), was used as the test organism. Artificial seawater was taken in the small tank and Shrimp eggs were added to one side of the tank and then that side was covered. The tank was kept under constant aeration for 48 hrs to hatch the Shrimp and to be matured as nauplii. The hatched Shrimps were attracted to the lamp through the perforated dam and with the help of a Pasteur pipette 10 living shrimps were added to each of the test tubes containing 5 ml of Brine solution.

Preparation of extract solution

Measured amount of each sample was dissolved in 1mL of DMSO. A series of solutions of lower concentrations were prepared by serial dilution with salt water. Then the total volume is adjusted upto 20 mL by adding salt water. Then the concentration of each mother solution will be 400µg/1mL.

Preparation of test solutions

Eight test tubes were taken for one mother solution and marked by desired concentrations. Then sufficient volume of mother solution was in these test tubes to make desired concentrations. Then 10 napulii were taken in each test tube and the volume of each test tube was adjusted upto 5 mL with salt water. Then the final concentration of the test tubes become 1µg/mL,5 µg/mL, 10 µg/mL,25 µg/mL,50 µg/mL,100 µg/mL,200 µg/mL and 400 µg/mL.

Preparation of Controls

Tamoxifen BP 20mg served as the positive control. From Tamoxifen tablet serial dilutions were made to get 1 µg/ml, 5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml. The control groups containing 10 living Brine Shrimp nauplii in 5 ml simulated seawater received the positive control solutions.

As for negative control, 50 and 125 µl of DMSO was added to each of the pre-marked test tubes and 10 Shrimp nauplii were taken in each test tube adjusted upto 5 ml with the help of simulated seawater. The test was considered invalid if the negative control showed a rapid mortality rate and therefore has to conduct again. The test tubes (containing nauplii) were then maintained at room temperature for 24 hrs under the light for observing the survival rate.

Counting of nauplii and analysis of data

After 24 hours, the test tubes were inspected using a magnifying glass and the number of survivors was counted. The percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed by using Microsoft Excel. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC50) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period. However, LC90 values were also calculated in the similar way for all fractions and the reference cytotoxic drug vincristine sulphate.

Tests for antimicrobial activity

The antimicrobial activity of the plant extract was performed by disc diffusion[10] method which is a widely accepted in-vitro investigation for preliminary screening of test agents that may possess antimicrobial activity.

Principle

In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media (Barry, 1976). The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter.[11]

Materials

Microorganisms

The microorganisms used in the antimicrobial activity assay of the extracts were carried out on both gram-positive and gram-negative bacteria. The organisms were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka.

Table: List of microorganisms on which the antimicrobial test was performed.

Name of bacteria on which crude is tested	
Escherichia coli	B.sabila Candida
albican	D. saprophyty Salmonela typhi
Staphylococcus aureus	Streptococcus pylori
P.mitu	Shigella bodyli
Body sentry	Vibrio parahemolisi

Culture media and chemicals

- Nutrient agar media
- Ethanol
- Chloroform

Equipments

- Filter paper discs
- Petridishes
- Inoculating loop
- Sterile cotton
- Sterile forceps
- Spirit burner
- Screw cap test tubes
- Nose-mask and Hand gloves
- Laminar air flow hood
- Autoclave
- Incubator
- Refrigerator

Test materials

The different crude extract by different solvents of the leaf extracts of Belamcanda chinensis are tested against the gram-positive and gram-negative bacteria listed in the Table.

Methods

Culture preparation

Composition of culture media

The following media are used normally to demonstrate the antimicrobial activity and to make subculture of the test organisms.

a. Nutrient agar medium

Ingredients	Amounts
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto yeast extract	1.0 gm
Bacto agar	2.0 gm
Distilled water q.s. to	100 ml
PH	7.2 □ 0.1 at 25 0C

b. Nutrient broth medium

Ingredients	Amounts
Bacto beef extract	0.3 gm
Bacto peptone	0.5 gm
Distilled water q.s.to	100 ml
PH	7.2 □ 0.1 at 250C

c. Mueller-Hinton agar medium

Ingredients	Amount
Beef infusion	30 gm
Casamino acid	1.75 gm
Starch	0.15 gm
Bacto agar	1.70 gm
Distilled water q.s.	100 ml
pH	7.3 □0.2 at 250 C

Preparation of culture media

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 250C) was adjusted at 7.2 ± 0.1 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15 lbs pressure/sq. inch at 1210C for 20 min. The slants were used for making fresh culture of bacteria that were in turn used for sensitivity study.

Sterilization procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petridishes and other glasswares were sterilized by autoclaving at a temperature of 1210C and a pressure of 15 lbs/sq. inch for 20 min. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Figure: Laminar Hood.

Preparation of subculture

In an aseptic condition under laminar air hood cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 h at 370C for their optimum growth. These fresh cultures were used for the sensitivity test.

Figure: Incubator.

Preparation of the test plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an

aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

Placement of disc and Incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 40C for about 24 h. Finally the plates were kept in an incubator at 370C for 24 h.

Determination of zone of inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

RESULTS AND DISCUSSION

Pharmacological Investigation of Belamcanda chinensis

DPPH radical scavenging assay

When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolourised, which can be quantitatively measured from the changes in absorbance. The IC50 values of the different fractions are presented in the Table 4.3. Percent scavenging of DPPH radical was found to rise with increasing concentration of the different fractions with highest scavenging displayed by crude of the plant.

Table. Absorbance and % inhibition of DPPH for Ascorbic Acid.

Test tube no.	Conc.(µg/ml)	Absorbance ofBlank	% of inhibition of Ascorbicacid	IC 50Value
1	25	0.271	31.36	132.324
2	50	0.271	38.74	
3	100	0.271	52.02	
4	200	0.271	63.73	
5	400	0.271	77.8	

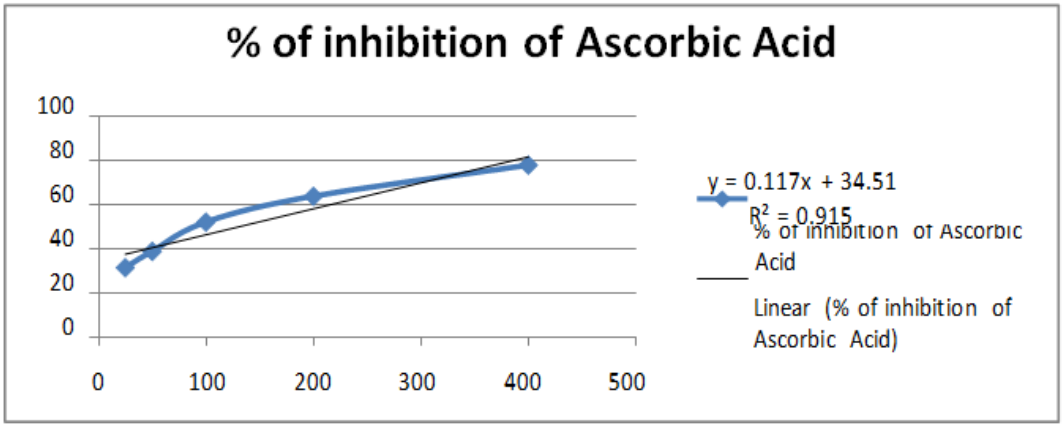


Figure: Graph for Absorbance and% inhibition of DPPH for Ascorbic Acid.

Table: Percent inhibition of the different fractions of the aerial parts of Belamcanda chinensis.

Sample	Concentrations	% of inhibition
Crude extract of aerial parts in methanol	25	5.26
	50	12.17
	100	24.01
	200	40.46
	400	82.57
Crude extract of aerial parts in ethyl acetate	25	9.87
	50	16.12
	100	19.41
	200	27.30
	400	47.70

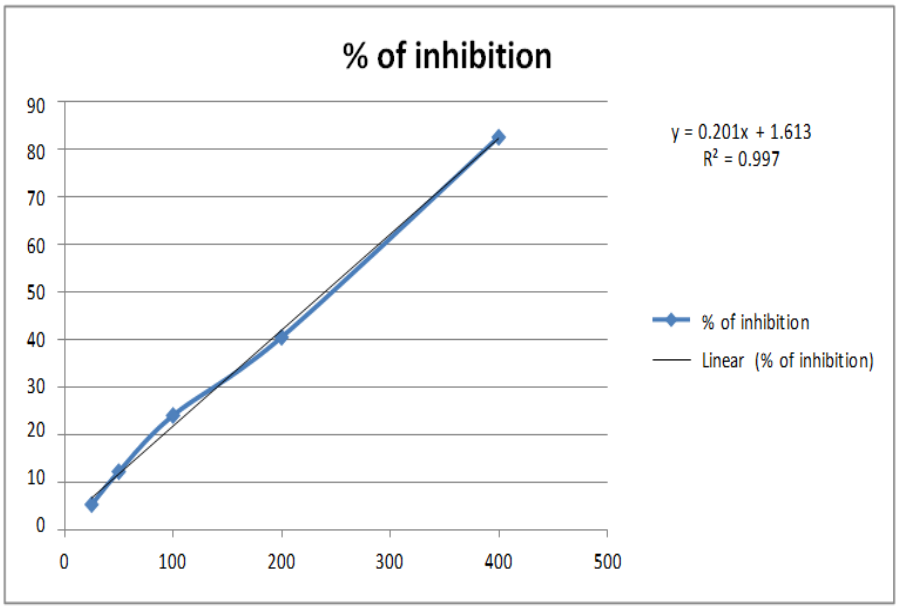


Figure: Graph for crude extract of Belamcanda chinensis aerial parts in methanol.

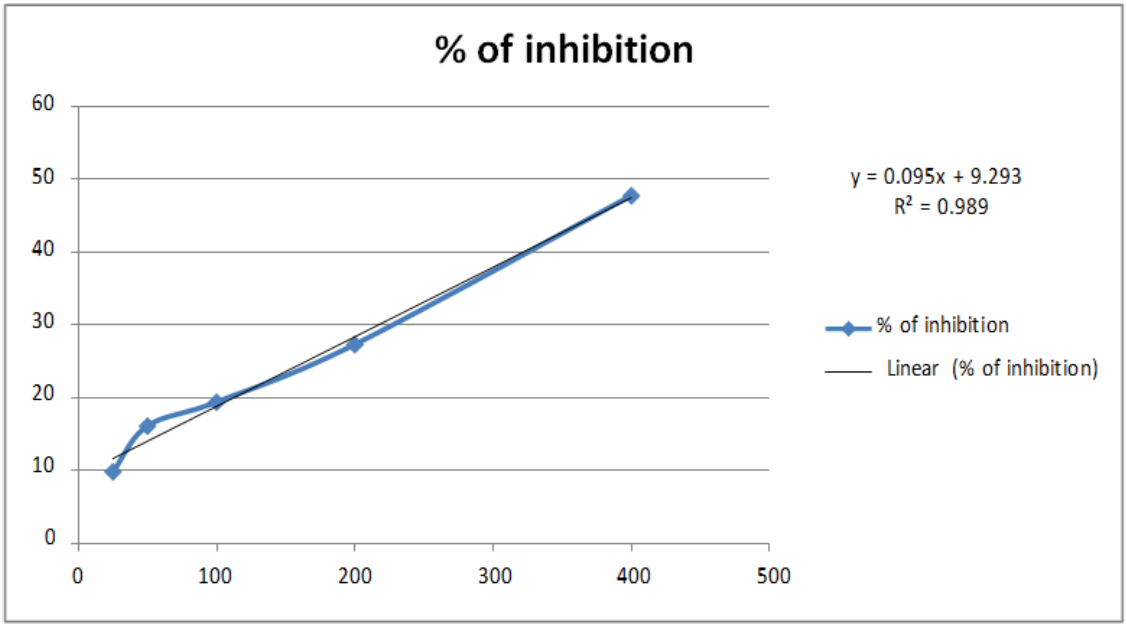


Figure: Graph for crude extract of Belamcanda chinensis aerial parts in Ethyl acetate.

Table: IC50 values of the different fractions of the aerial parts of Belamcanda chinensis.

Sample/Standard	IC50 (g/ml)
Standard	132.324
BCAE	240.73
BCAM	428.49

Here, BCAE=Belamcanda chinensis aerial parts in Ethyl acetate, BCAM= Belamcanda chinensis aerial parts in Methanol.

Brine Shrimp lethality bioassay for cytotoxic activity

All the partitionates were also subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this study, methanol and ethyl acetate soluble fractions were found to be highly toxic to Brine Shrimp nauplii, with LC50 of 16.218µg/ml and 0.048µg/ml respectively while the LC50 of the reference anticancer drug tamoxifen BP 20mg was 0.003µg/ml. All fractions produced concentration dependent increment in percent mortality of Brine Shrimp nauplii indicating the presence of cytotoxic principles in these extractives.

Table: Brine Shrimp lethality bioassay of the different fractions of Belamcanda chinensis.

Test solution	Conc. (µg/ml)	Log Conc.	% Mortality	LC50 (µg/ml)	LC90 (µg/ml)
Methanol extract	1	0	20	16.218	234.423
	5	0.69897	30		
	10	1	30		
	25	1.39794	50		
	50	1.69897	60		
	100	2	90		
	200	2.30102	90		
	400	2.60205	100		
Ethyl Acetate extract	1	0	70	0.048	44.668
	5	0.69897	70		
	10	1	80		
	25	1.39794	90		
	50	1.69897	100		
	100	2	90		
	200	2.30102	100		
	400	2.60205	100		
Standard	1	0	70	0.003	42.658
	5	0.69897	90		
	10	1	90		
	25	1.39794	100		
	50	1.69897	80		
	100	2	80		
	200	2.30102	100		
	400	2.60205	100		
	1	0	70		

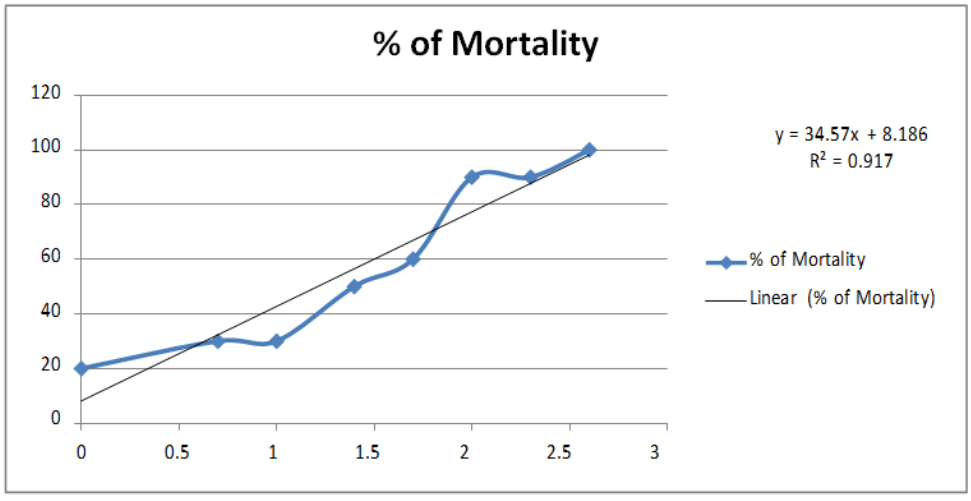


Figure: Graph for crude extract of Belamcanda chinensis aerial parts in methanol.

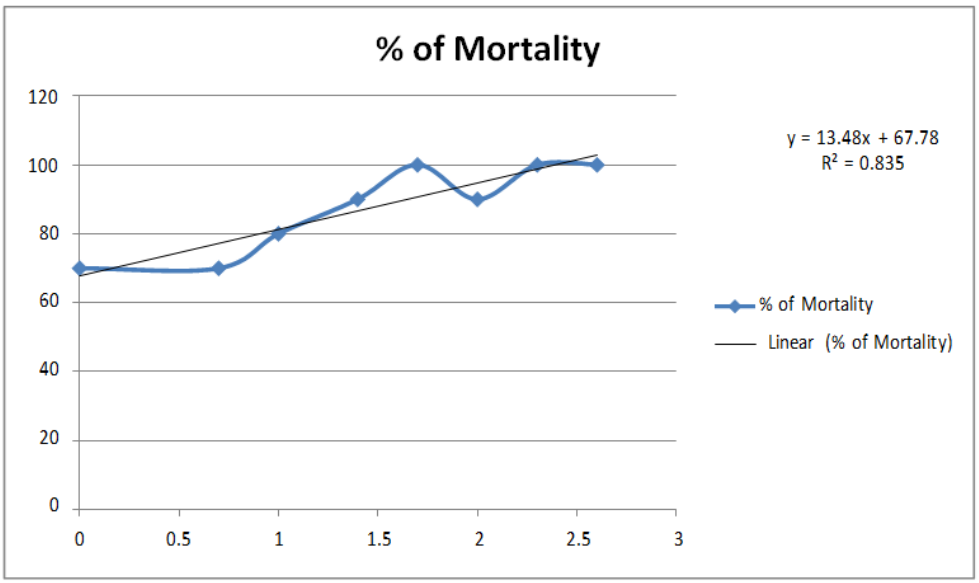


Figure: Graph for crude extract of Belamcanda chinensis aerial parts in Ethyl acetate.

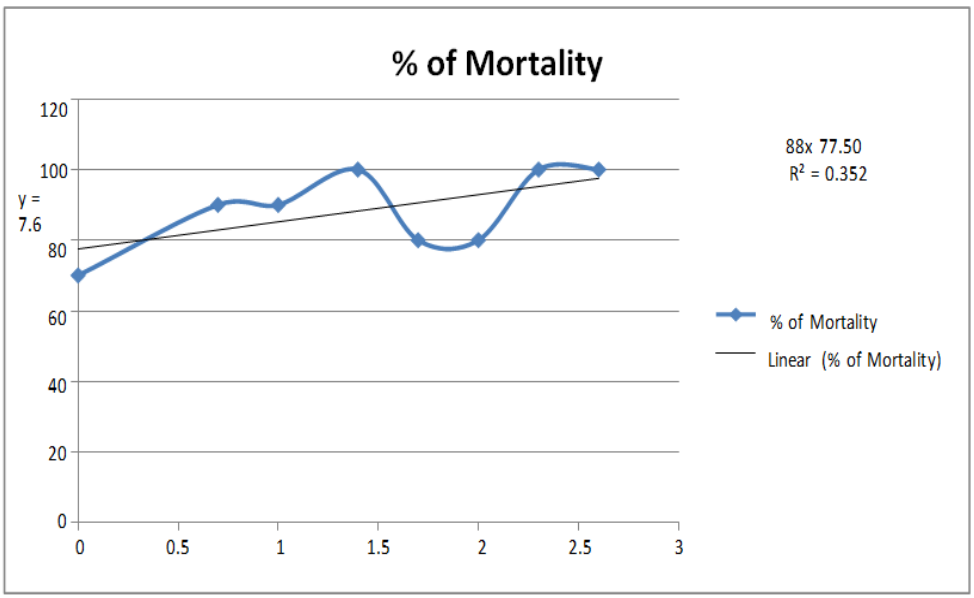


Figure: Graph for Standard.

Table: Results of different crude from different solvent of the aerial parts of Belamcanda chinensis in Brine Shrimp lethality bioassay.

Sample	Regression Equation	R^2
BCAM	y = 34.57x +8.186	0.917
BCAE	y = 13.48x + 67.78	0.835
Standard	y = 7.688x+77.50	0.352

Here, BCAE=Belamcanda chinensis aerial parts in Ethyl acetate, BCAM= Belamcanda chinensis aerial parts in Methanol.

Antimicrobial Activity of Belamcanda chinensis

Antimicrobial activity of leaves of Belamcanda chinensis at a concentration of 0.01mg/10ml with solvent ethanol. (Plant extract).

Table: Antimicrobial activity of Belamcanda chinensis aerial parts.

Name of microorganism	Zone(cm) of inhibition of aril parts of MABC	Zone(cm) of inhibition of aerial parts of EABC	Zone(cm) of inhibition of Standard
Shigellabodyli	0.6	0.5	3.5
Vibrio parahemolyticus	-	0.5	4
Candida albicans	-	0.6	4
D. saprophyty	1.7	0.5	4
B.sabila	-	1	3.5
Salmonela typhi	-	-	3.5
Staphylococcus aureus	0.8	0.5	3.8
Escherichia coli	1	1	3.5
Streptococcus pylori	0.9	0.7	3.5

DISCUSSION

According to the result this plants aerial parts contain compounds which can give antioxidant activity like isorhapontigenin.[12] In DPPH the fractions of the aerial parts of Belamcanda chinensis showed dose dependent scavenging of DPPH.DPPH radical scavenging is a popular and reliable method for screening the free radical scavenging activity of compounds or antioxidant capacity of plant extracts.[13] When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolourised, which can be quantitatively measured from the changes in absorbance. The IC50 values of the different fractions are presented in the Table 4.2.Percent scavenging of DPPH radical was found to rise with increasing concentration of the different fractions with highest scavenging displayed by crude of the plant. Ethyl acetate extracts exhibited moderate DPPH free radical scavenging effects. The role of these plant extracts in the traditional treatment in humans is highlighted.[14]

According to the result of cytotoxicity test, these plants aerial parts contain compounds which can give anticancer activity. The value of IC50 of the aerial parts of this plant gives almost similar result with the standard. The IC50 values of the different fractions are presented in the Table. The value totally depends on the dose range. They show their activity according to their dose range same as standard. An activity-directed fractionation and purification process was used to isolate antitumor compounds from the aerial parts of Belamcanda chinensis(L.) DC. The ethyl acetate extract showed greater antitumor activities than the other extracts,

consequently leading to the isolation of 18 compounds identified a-sitosterol (1), dausterol (2), quercetin (3), kampferol (4), shikimic acid (5), gallic acid (6), ursolic acid (7), betulin (8), betulonic acid (9), betulone (10), tectoridin (11), irisflorentin (12), 4′,5,6-trihydroxy-7-methoxyisoflavone (13), tectorigenin (14), irilins A (15), iridin (16), irigenin (17), and iristectongenin A (18). Compounds 3–10, 13, and 15 were isolated from Belamcanda chinensis for the first time. Compounds 4 and 7–10 showed potent cytotoxic activities against PC3, MGC-803, Bcap-37, and MCF-7 cell lines. The mechanism of the antitumor action of compound 7 was preliminarily investigated through acridine orange/ethidium bromide (AO/EB) staining, Hoechst 33258 staining, and terminal deoxynucleotidyltransferased UTP nick end labeling (TUNEL) assay, which indicated the growth inhibition of MGC-803 cells via the induction of tumor cell apoptosis.[14] Based on result aerial parts of Balamcanda chinensis have very low or moderate antimicrobial property. The antimicrobial activity of aerial parts of this plant is presented in the Table.

Tectorigenin (present in very little content in aerial parts) was found to have strong antifungal activity against dermatophytes of the genera Trichophyton with MIC ranging from 3.12-6.25ml.[11]

CONCLUSION

According to the results, it can be proposed that the aerial parts of Belamcanda chinensis in general; methanol and ethyl acetate soluble fractions of aerial parts in particular, have antioxidant, cytotoxic, and very little antimicrobial properties. Again, these findings may justify scientifically the basis for the use of this plant in folk medicine for the treatment of a wide range of disease of different aetiology such as skin out-growth, cancer, inflammation, insanity, diarrhoea etc. These results also lend support to the relevant phytochemical and pharmacological works carried out so far on Belamcanda chinensis. However, further studies are suggested to be undertaken to understand the underlying mechanism of the observed activities and to isolate, purify and characterise active phytochemical ingredient(s) responsible for these bioactivities in animal models.

FUTURE DIRECTIONS

Natural products, especially those of plant origin, have been a promising source of new lead compound for drug discovery for ages. Bangladesh is blessed with rich floristic resources, where a large number of plants still remain unexplored. So well designed, systematic and

objective research in this area might benefit our people who have been deluged with superfluity of diseases, and who lack technological and economic resources to cope up with them with orthodox medicine.

The future goal of this study is to identify effective, cheap and available modalities to cope up with the upsurge of the dangers of diseases of different aetiology in Bangladesh. Approaches may be developed to prevent and/or treat illness easily and effectively with readily available and cheaper resources. This research may be a platform for further investigation in this area. It is likely to show directions for the researchers to find ways out to save our lay people from the curse of diseases. Future endeavours in this area may open up exciting new therapeutic avenues.

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