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Research Article

Investigation of *In vitro* Antioxidant, Antimicrobial and Cytotoxic activity of *Diplazium esculentum* (RETZ). SW.

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ABSTRACT

Diplazium esculentum (Retz). Sw. leaf is traditionally used in headache, pain, fever, wounds, dysentery, glandular swellings, diarrhea and various skin infections. This study investigated the antioxidant, antimicrobial and cytotoxic properties of the leaf of *D. esculentum* using different in vitro experimental models. The powdered leaf of *D. esculentum* was extracted using chloroform (DECH) and methanol (DEM). The antioxidant activities of these extracts were then determined by DPPH radical scavenging activity assay, Cuprac reducing antioxidant activity assay and total antioxidant capacity assay. Antimicrobial activity was determined by disc diffusion method and the minimum inhibitory concentration (MIC) was determined by broth dilution assay against 3 gram positive and 5 gram negative bacteria. Brine shrimp lethality bioassay was also performed to evaluate the cytotoxic potential of the extracts. The phenol and flavonoid content and total antioxidant activity of the extracts were moderate in comparison with the standard. The DPPH radical scavenging capacity was low compared to the standard. However, DECH and DEM showed strong antioxidant activity in cupric ion reducing capacity assay. The plant extracts possess strong antimicrobial activity and the order of zone of inhibition observed by the DECH was: *Sarcina lutea* (18.67 mm) > *Salmonella typhimurium* (16.33 mm) > *Bacillus subtilis* (15.33 mm), *Klebsiella pneumoniae* (15.33 mm) > *Shigella boydii* (14.67 mm) > *Escherichia coli* (12.33 mm) > *Staphylococcus aureus* (11.33 mm) > *Vibrio cholerae* (10.67 mm). MIC values of DECH and DEM were between 1.6 - 12.5 mg/ml. The lowest MIC value was 1.6 mg/ml shown by DEM against *Salmonella typhimurium* and *Bacillus subtillis*. The largest MIC value was 12.5 mg/ml showed by both extracts against *Klebsiella pneumoniae*. The results of the present study suggest that the leaf of *D. esculentum* possess significant cytotoxic, antimicrobial and antioxidant properties.

Keywords: *Diplazium esculentum*, Antioxidant, Antimicrobial, cytotoxic, medicinal plant.

INTRODUCTION

Medicinal plants containing various chemical substances have been used as potential therapeutic agent throughout history. The presence of pharmacologically active secondary metabolites in plants rationalizes the use of plant extracts as a therapy in different diseases including infectious diseases[1]. As microbial resistance has been emerging, plant secondary metabolites could be promising candidates for developing potential new antimicrobial agents[2].

Besides, scientific studies on the contribution of reactive oxygen species (ROS) in different diseases excelled the quest for natural antioxidants. As a result, phytochemicals in vegetables have drawn the attention on their role in preventing diseases caused by ROS. These radicals are involved in many diseases like cardiovascular malfunctions, DNA damage, tissue injury, and tumor promotion[3,4]. When the balance between ROS production and antioxidant defenses is disturbed, oxidative stress develops[5].

Excessive oxidative stress imposes a risk to human health with a good correlation to degenerative diseases, chronic inflammatory processes[6], cardiovascular diseases and cancer[7]. Previous studies have established that ROS can cause tissue damage and act as signaling molecules in the pathogenesis of inflammation and carcinogenesis. Antioxidants could prevent accumulation of these ROS and can be used for the treatment of these diseases[8]. Therefore, there has always been a particular interest in finding natural antioxidants from plant materials. Vegetables consumed either as fresh or cooked could be a great source of natural medicines along with their role as potential nutrients such as carbohydrate, protein, dietary fiber, vitamins and minerals, they are also known for their therapeutic value.

Diplazium esculentum (Retz.) Sw. is a common pteridophytes from the family Athyriaceae. It is locally known as 'Dheki Shak' and distributed widely in Bangladesh. It is considered as high economic value fern amongst the natives of Bangladesh. This plant can attain an average height of 0.5 to 2.5m. The dried rhizomes are used as insecticides and decoction is used to cure haemoptysis and cough[9]. Besides, the tribal communities, ethnic groups and folklore throughout the world are utilizing various parts of this plant like rhizome, stem, fronds, pinnae and spores in various ways for the treatment of various ailments since ancient time. Mainly the leaves are used in headache, pain, fever and wounds[10,11]. This plant is also used in dysentery, glandular swellings, indigestion, diarrhea and various skin infections[12]. The plant contains steroids, triterpenoids, phenols, flavones, flavonoids such as myrcetin and alpha-tocopherol[13,14]. Pharmacological properties such as laxative[15], anti-inflammatory[11,16], antioxidant[17], anthelmintic[18], antimicrobial[19], cytotoxic[19] activities have been reported. The traditional use of *D. esculentum* in different infectious and inflammatory diseases and lack of scientific study evaluating its traditional uses have convinced us to design the present study to evaluate the antimicrobial , antioxidant and cytotoxic activity of the leaf of *D. esculentum*.

MATERIALS AND METHODS

Plant materials and extract preparation

Leaf of *D. esculentum* was collected from Tangail district of Bangladesh in December, 2012. The collected sample was identified by the experts of National Herbarium, Mirpur, Dhaka, Bangladesh (Accession No. 37939) where a voucher specimen has been deposited for further reference. The leaves were then washed, dried and grounded and 400 g of the dried powder was taken in two different beakers in equal amount. The samples were macerated with 450 ml chloroform and 450 ml methanol for seven days with occasional stirring. Then they were filtered, dried and the yield was 12% and 18% respectively .The percentage extract yield was estimated using the formula: Dry weight/Dry material weight x 100[20].

Chemicals

Sodium nitroprusside was obtained from BDH Chemicals Ltd., Poole, England; Griess reagent from Roch-light Ltd., Suffolk, England; Sodium Phosphate (Na3PO4) from Merck, Mumbai, India; Ammonium Molybdate from Merck DGaA, Germany; Quercetin from Sigma Chemicals, USA; Ascorbic acid from SD Fine chem. Ltd., Biosar, India. 1, 1-Diphenyl-2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma Chemical Co. (St., Louis, USA). Gallic acid, Folin Ciocalteu reagent, and methanol were purchased from Merck Co. (Germany).

Microorganisms

Pure culture of *Escherichia Coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Salmonella typhimurium*, *Sarcina leutea*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Shigella boydii* were obtained from the Microbiology laboratory of Department of Pharmacy, Primeasia University, Dhaka, Bangladesh (106 CFU ml^{-1}).

Phytochemical screening

The crude extracts of *D. esculentum* were tested for the detection of the presence of plant secondary metabolites following standard procedures[21].

Determination of Total Polyphenol content

The content of total phenolic compounds in the extracts was determined by Folin-Ciocalteu reagent[22]. 1 ml of extract (200 µg/ml) was mixed with 500 µl of Folin- Ciocalteu reagent and 4 ml of 7.5% sodium carbonate solution. The mixture was then incubated for 1 h at 20°C. The absorbance of the solution was measured at 765 nm against blank. The total content of phenolic compounds was calculated in gallic acid equivalents (GAE) with the formula: $A = (C \times V) / m$; where A is the total content of phenolic compounds, mg/g plant extract in GAE; C is the concentration of gallic acid obtained from the calibration curve, mg/ml; V is the volume of extract in ml and m is the weight of plant extract in g.

Determination of total flavonoid content

The total flavonoid content of the crude extracts was determined using standard procedure[23]. 1 ml of extract solution (100 µg/ml) or quercetin (standard)

in different concentrations were mixed with 3 ml methanol, 200 µl (10%) aluminium chloride solution and 200 µl (1 M) potassium acetate solution. Then 5.6 ml of distilled water was added to the mixture and incubated for 30 min at room temperature. The absorbance of the solution was measured at 415 nm against blank. The total content of flavonoid compounds in plant extracts were calculated and expressed as mg of quercetin equivalents (QE) / gm of extract.

Determination of total antioxidant capacity

Total antioxidant capacities of the extracts were determined by the phosphomolybdenum method[24]. 0.3 ml of extract was mixed with 3 ml of reagent solution and were incubated at 95°C for 90 min. The reduction of Mo (VI) to Mo(V) by the extracts and the formation of a green phosphate/Mo(V) complex at acid pH forms the basis of this method. The reaction solution is then cooled to room temperature and the absorbance was measured at 695 nm against blank. The antioxidant activity of the extract was expressed as the number of equivalent of ascorbic acid.

DPPH Assay

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging by the extracts was determined by standard procedure[25]. 200 µl of the plant extract of different strength (5, 25, 50, 100, 500 µg/ml) was mixed with 2 ml of a 0.004% methanol solution of DPPH. After 30 min, the absorbance was determined at 517 nm using a UV spectrophotometer (Shimadzu, UV-1601PC) against a blank. Absorbance of DPPH solution without the extract or standard agent was used as control. The percentage scavenging activity of the extracts was calculated with the formula: % scavenging activity = {(A0 – A1) / A0} × 100; where A0 is the absorbance of the control and A1 is the absorbance of the extract or standard.

Cupric ion reducing antioxidant capacity assay

The cupric ion reducing capacities of the plant extracts were performed by standard method[26]. 1.0 ml of 0.01 M $CuCl_2.2H_2O$ solution was mixed with 500 µl of plant extract or standard of different concentrations (5, 25, 50, 100, 200 µg/ml). Then 1.0 ml of ammonium acetate buffer (pH 7.0) and 1.0 ml of 0.0075 M of neocaproine solution was mixed. Then 600 µl of distilled water was added and the final volume of the mixture was adjusted to 4.1 ml. The total mixture was then incubated for 1 h at room temperature. The absorbance of the solution was measured at 450 nm using UV spectrophotometer against blank.

Screening of antimicrobial activity

The disc diffusion method was used to test for antimicrobial activity of the plant extracts[27]. Briefly, the extract solution was prepared at a concentration of 300 µg/ml. Then the paper discs were impregnated by using the extract and placed onto the nutrient agar media inoculated with the test bacteria and incubated at 37^o C for 24 h. Azithromycin (30 µg/disc) (oxoid,UK) were used as positive control and blank discs (impregnated with solvents followed by evaporation) were used as negative control. After incubation the culture plates were examined and the zones of inhibition were measured in mm scale. For each test the mean of triplicate results were taken.

Determination of minimum inhibitory concentration (MIC)

Determination of the minimum inhibitory concentration (MIC) was carried out using the broth dilution method[28]. In brief 1.0 ml of the reconstituted extract solution at a concentration of 200 mg/ml was added to another test tube containing 1 ml of sterile broth so as to obtain a concentration 100mg/ml. 1 ml of this solution was transferred to another test tube till the 10th test tube was reached. The 11th test tube did not contain any extract, but a solution of pure solvent served as negative control. Then 1 ml of an 18 h old culture of each of the organisms earlier adjusted at 106 CFU/ml was put into each tube and thoroughly mixed. The tubes were incubated at 37^o C for 24 h and observed for growth of bacteria in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC.

Cytotoxic activity

The cytotoxic activity of the crude extracts was investigated using Brine shrimp lethality bioassay[29]. *Artemia salina* leaches were collected and taken in a small tank of water at 37^{o}C with constant oxygen supply to allow hatching of the shrimps for two days and to mature as nauplii. 5 ml of seawater was taken in each vial. Specific volume of the test samples were taken and dissolved in specific volume of dimethyl sulfoxide (DMSO) to get stock solutions. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method and was added to the vials. The concentrations of the obtained solutions in the vials were as 1 µg/ml, 5 µg /ml, 10 µg /ml, 20 µg /ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 500 µg/ml. 10 nauplii were added by micropipette to each of the vials. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted and the percentage of

lethality of the brine shrimp nauplii was calculated for each concentration.

Statistical analysis

Analyses were performed in duplicates for antioxidant studies and triplicates for antimicrobial study. Data were presented as mean ± SD. SPSS 11.5 was used to calculate the LC_{50} in cytotoxicity study using probit chart.

RESULTS

Phytochemical screening

Phytochemical screening revealed the presence of various plant secondary metabolites listed in table 1 in the DEM extract of leaves of *D. esculentum* while the DECH extract gets no result for Carbohydrate, tannin and alkaloid.

Total phenol, flavonoid content and total antioxidant activity

Table 2 shows the total phenol, flavonoid and total antioxidant activity of the DECH and DEM extracts. The phenol contents of DECH and DEM is low compared to standard, the flavonoid content of DECH and DEM is high and the total antioxidant capacity (TAC) of two extracts DECH and DEM showed higher antioxidant activity compared to standard Ascorbic Acid at all the concentrations.

DPPH radical scavenging activity

Table 3 shows the free radical scavenging activity of DECH and DEM. The IC_{50} of DECH and DEM were 95669.52 and 5907.53 while the IC_{50} of the standard antioxidant Ascorbic acid was 13.76. In terms of DPPH radical scavenging activity it can be said on the basis of the results that the plant extracts had lower activity compared with the standard.

Cupric ion reducing capacity

Cupric ion reducing capacity of DECH and DEM was investigated and compared with standard Ascorbic acid (AA). At concentrations of 5, 25, 50, 100, 200 µg/ml, the absorbance of the two extracts showed steady increase in their absorbance and the trend is similar to AA (Figure 1). As the increase in absorbance indicates increased reducing capacity so it can be said that the plant extractives have significant antioxidant activity and the activity increased with the increasing concentration of the extracts.

Antimicrobial Activity

The antimicrobial activity of DECH and DEM extracts of *D. esculentum* leaves is shown in Table 4 in a comparative way with standard antibiotic disc Azithromycin (30 µg/disc). The present study revealed that the zone of inhibition observed by DECH and DEM is almost similar and they didn't vary significantly. The antimicrobial activity is significant compared to standard. The highest zone of inhibition is observed for *Sarcina lutea* of 18.67 mm followed by *Salmonella typhimurium* (16.33 mm). On the other hand, in DEM the highest zone of inhibition is found from *Samonella typhimuriun* 16.67 mm followed by *Klebsiella pneumonia* 15.33 mm. The order of zone of inhibition of DECH is *Sarcina lutea* (18.67 mm) > *Salmonella typhimurium* (16.33 mm) > *Bacillus subtilis* (15.33 mm), *Klebsiella pneumoniae* (15.33 mm) > *Shigella boydii* (14.67 mm) > *Escherichia coli* (12.33 mm) > *Staphylococcus aureus* (11.33 mm) > *Vibrio cholerae* (10.67 mm). And the order of zone of inhibition for DEM is *Bacillus subtilis* (19.67 mm) > *Salmonella typhimurium* (16.67 mm), *Shigella boydii* (16.67 mm) > *Klebsiella pneumoniae* (15.33 mm) > *Sarcina lutea* (14.67mm) > *Escherichia coli* (13.67 mm), *Vibrio cholerae* (13.33mm) > *Staphylococcus aureus* (11.33 mm). All the extracts were found very effective against both *Salmonella typhimurium* and *Sarcina lutea*. The extracts showed relatively lower antimicrobial activity against *Klebsiella pneumoniae*. Positive control /standard Azithromycin produced significant zones of inhibition against all the tested bacteria while no zone of inhibition was observed by the negative control which implies that the solvents have no involvement in the antimicrobial activity of the extracts.

Minimum inhibitory Concentration (MIC)

The result of minimum inhibitory concentration assay is shown in table 5. MIC values of DECH and DEM extracts were found between 1.6 -12.5 mg/ml. The best MIC value was 1.6 mg/ml obtained by DEM against *Salmonella typhimurium* and *Bacillus subtillis*. The lowest MIC value was 12.5 mg/ml obtained by both extracts against *Klebsiella pneumoniae*.

Cytotoxic activity

In Brine shrimp lethality bioassay both extracts produced concentration dependent increment in percent mortality of Brine Shrimp nauplii indicating the possible presence of cytotoxic principles in these extracts (Figure 2). The LC_{50} values of DECH and DEM were 1.87 µg/ml and 1.62 µg/ml compared to the LC_{50} of standard anticancer drug Vincristine Sulphate 0.66 µg/ml (Table 6).

DISCUSSION

The antioxidant activity of the extracts were found in

different methods which indicates the involvement of multiple mechanisms. The total antioxidant potentials of DECH and DEM were calculated from their ability to reduce the reduction of Mo (VI) to Mo (V) and formation of a green phosphate /Mo (V) complex at acid pH. All the extracts showed good total antioxidant activity. The antioxidant capacity of the extracts may be due to the presence of the phenolics and flavonoids which is also supported by the phytochemical screening showing positive results for the presence of phenols and flavonoids[14,30].

DPPH free radical scavenging is an well accepted method by which antioxidant properties of plant extractives can be evaluated .The effect of antioxidants on DPPH radicals is believed to be due to their hydrogen donating ability and inhibiting lipid peroxidation[31]. Free radical scavenging is a significant feature to various plants extracts which is very important to prevent the detrimental involvement of free radicals in various diseases like cancer[32]. The IC_{50} values of DECH and DEM is far greater than the standard Ascorbic acid which indicates the involvement of other mechanisms other than DPPH radical scavenging activity of the antioxidant activity of *D. esculentum*.

Poly phenols are major plant compounds having antioxidant activity which have been claimed to have anticancer activity[33].This activity is thought to be mainly due to their redox properties which play an important role in absorbing and neutralizing free radicals, receiving singlet and triplet oxygen or decomposing peroxides. Phenolics are important components of plant and many of the pharmacological effects exerted by plant could be attributed to the presence of valuable constituents contained in plant[34]. The results of the present work suggest that strong antioxidant activity of plant material thus can be correlated with a good phenolic content.

Flavonoids are polyphenols commonly found in fruits, vegetables and plants. Flavonoids contribute significantly as natural antioxidant. Flavonoids have been shown to possess antimutagenic and antimalignant effect along with its role in preventing diseases like cancer and inhibiting low density lipoprotein (LDL) oxidation induced by free radicals. Flavonoids have also shown activity as antibacterial, antiviral, antitumor and anti-inflammatory activities[32,35,36]. Flavonoids have also shown activity as a chemopreventive agent through their effect on signal transduction in cellular proliferation and angiogenesis[37]. Results of the present study revealed that the level of flavonoid content is significant in DECH and DEM extracts (Table 1) of *D. esculentum*.

Cupric ion reducing capacity assay is a simple method for the antioxidant capacity assay for dietary polyphenols, vitamin C and vitamin E which utilizes the copper (II)- neocuproine (Cu(II)-Nc) reagent as the chromogenic oxidant[38].The color was due to the Cu (I)-Nc chelate formed. The CUPRAC absorbance of the extracts could be due to the reduction of Cu (II)-neocuproine reagent by the hydroxylated probe which may be decreased in the presence of .OH scavengers and the difference being proportional to the scavenging ability of the tested samples[39]. The DECH and DEM extracts showed strong reducing activity compared to the standard ascorbic acid.

Various studies have conducted in the last five decades focusing on the phytoconstituents and antimicrobial properties of herbs, plants, spices and their derivatives. As a result, several drugs have been developed including quinine, emetin, belladonna, amongst others[28]. Previous workers have suggested the existence of a direct relationship between the chemical structure of a bioactive compound in plant extracts and antimicrobial activity[28,40] which was further supported by the concept that extracts of some plants inhibited the growth of various microorganisms at different concentrations[41]. The antibacterial activity is believed to be due to the presence of some bioactive compounds like alkaloids, tannins, saponins, terpenes and flavonoids[42,43]. In connection to this our present study revealed the presence of several secondary metabolites in the leave extracts of *D. esculentum* which may be correlated to their antimicrobial activity. The diameter of inhibition zone may be varied due to various strains and species of bacteria[44].The data obtained in this study showed that the highest zone of growth inhibition was observed by DEM extract against *Bacillus subtilis* (19.67 mm) while the least activity was shown by the DECH extract against *Vibrio choleare* (10.67 mm). The two extracts DECH and DEM showed almost similar pattern of inhibition zones against the organisms tested. Infectious diseases impose a big threat to public health in developing countries which may be due to the unavailability and high cost of medicines[45]. Recently, there has been growing interests in plants as sources of antimicrobial agents due to their use from ancient time and a good portion of the world's population, particularly in developing countries, depends on plants for the treatment of infectious and non-infectious diseases[46]. Determination of the MIC is an effective way to measure the effectiveness of antimicrobial agents which is predictive of therapeutic outcome. MIC and antimicrobial activity shares inversely proportional relationship, as agents with lower activity against a particular organism

usually gives higher MIC, while a highly reactive agent gives lower MICs. The MIC values of the DECH and DEM extracts of the leaves of *D. esculentum* are shown in Table 4. The MIC values are in the range of 1.6 mg/ml to 12.5 mg/ml. The highest concentration required to inhibit the growth was 12.5 mg/ml for both DECH and DEM against *Klebsiella pneumoniae*. The lowest concentration to inhibit the growth was 1.6 mg/ml for *Bacillus subtilis* and *Salmonella typhimurium* by the DEM extract. It can be seen from the table that the MIC values for DECH and DEM extracts followed similar pattern. In this study, the MIC values also support the results obtained in the antibacterial screening. Conventional antibiotics records the MIC values in the range of 0.01 - 10 µg/ml[28] which are in a pure form, on the contrary the crude plant extracts of the present work may contain some sort of impurities that may be inert and do not have any antibacterial activities.

The brine shrimp lethality represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties[47]. In the present study the brine shrimp lethality of DECH and DEM extracts of *D. esculentums* was determined .The LC_{50} values of the extracts and standard anticancer drug Vincristine Sulphate are given in Table 6. Both of the extracts showed strong cytotoxic activity compared to the standard. The degree of lethality was found to be directly proportional to the concentration of the extract. Highest rates of mortalities took place at a concentration of 500 µg/ml whereas least mortalities were observed at 1 µg/ml concentration (Figure 2). The LC_{50} values of the plant extracts were obtained by plotting the percentage of the shrimp nauplii killed against the concentrations of the extracts and the best fit line was obtained from the data by means of regression analysis. This significant lethality of plant extracts to brine shrimp is an indicative of the presence of potent cytotoxic components which warrants further investigation. The brine shrimp lethality bioassay can detect a board spectrum of bioactive principles in crude samples for the front-line screening. This method is very useful and has been used in predicting cytotoxic and antitumor activity of extracts[48].The phytochemicals present in the extracts such as alkaloids, steroids may be responsible for the cytotoxic activity of extracts[49].The cytotoxic activity of DECH and DEM extracts further supports the antibacterial activity of the extracts.

CONCLUSION

The findings of this study highlighted several novel aspects of *D. esculentum* derived extracts with regard to their antioxidant, antimicrobial and cytotoxic effects. The present study indicated that the leaf extracts of *D. esculentum* possesses significant antimicrobial activity and cytotoxic activity. Moreover the antioxidant potential of the plant in case of total antioxidant activity, flavonoid content and cupric ion reducing capacity exhibited strong antioxidant activity. It is evident that the leaf of *D. esculentum* can be used as a natural antioxidant, antimicrobial and cytotoxic agent. However further investigation is required to isolate and characterize the active chemical constituents responsible for the given activities.

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Table 1

Results of phytochemical screening of DECH and DEM extracts of *D. esculentum*.

Extract	Carbohydrate	Glycoside	Saponin	Steroid	Alkaloid	Glucoside	Tanin	Flavonoid
DECH	-	+	+	+	-	+	-	+
DEM	+	+	+	+	+	+	+	+

'+ve' sign indicates the presence and '–ve' sign indicates the absence.

Table 2
Total amount of plant phenolics, flavonoids and total antioxidant capacity *of* DECH and DEM of *D. esculentum.*

Extracts	Total phenol (in mg/g , Gallic acid equivalents)	Total flavonoid (in mg/g, Quercetin equivalents)	Total antioxidant (in mg/g, Ascorbic acid equivalents)
DECH	20.11	137.45 ± 11.54	207.41 ± 2.62
DEM	45.29	161.17 ± 12.63	181.94 ± 5.89

*Results are expressed as mean of duplicate experiments ± SD (standard deviation)

Table 3
DPPH radical scavenging activity of DECH and DEM extracts of *D. esculentum.*

Extracts	IC_{50} (µg/mL)
DECH	95669.52
DEM	5907.53
Ascorbic Acid	13.76

Table 4
Antimicrobial activity of DECH and DEM extracts of *D. esculentum* using disk diffusion method.

	Diameter of inhibition Zone in mm scale		
Test organisms	**Azithromycin (30 µg/disc)**	**DECH (300 µg/disc)**	**DEM (300 µg/disc)**
Escherichia coli	38	12.33 ± 1.25	13.67 ± 2.05
Salmonella typhimurium	32	16.33 ± 1.25	16.67 ± 1.70
Bacillus subtilis	26	15.33 ± 1.25	19.67 ± 1.25
Shigella boydii	28	14.67 ± 0.94	16.67 ± 1.25
Vibrio cholerae	36	10.67 ± 0.94	13.33 ± 0.47
Klebsiella pneumoniae	40	15.33 ± 2.50	15.33 ± 2.50
Staphylococcus aureus	42	11.33 ± 1.70	11.3 ± 1.25
Sarcina lutea	38	18.67 ± 1.70	14.67 ± 2.05

***Data expressed as mean ± SD of triplicate experiments.**

Table 5
Minimum inhibitory concentrations (MIC) of DECH and DEM extracts of *D. esculentum.*

Test organisms	Minimum inhibitory concentrations (MIC) (mg/ml)	
	DECH	**DEM**
Escherichia coli	6.3	3.2
Salmonella typhimurium	6.3	1.6
Bacillus subtilis	3.2	1.6
Shigella boydii	3.2	3.2
Vibrio cholerae	3.2	3.2
Klebsiella pneumoniae	12.5	12.5
Staphylococcus aureus	6.3	6.3
Sarcina lutea	3.2	3.2

Table 6
Effects of DECH and DEM extracts of *D. esculentum* on brine shrimp nauplii.

Conc. (C) (µg/ml)	Log C	% Mortality		LC_{50} (µg/ml)		Vincristine Sulfate			
		DECH	DEM	DECH	DEM	Conc (C) (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)
1	0	0	0			0.08	-1.11	20	
5	0.7	7	7			0.16	-0.81	30	
10	1	10	10			0.31	-0.51	40	
20	1.3	13	13			0.63	-0.21	50	
50	1.7	17	17			1.25	0.10	60	
100	2	20	20	1.87	1.62	2.5	0.40	80	0.66
200	2.3	23	23			5	0.70	90	
500	2.7	27	27			10	1	100	
						20	1.30	100	

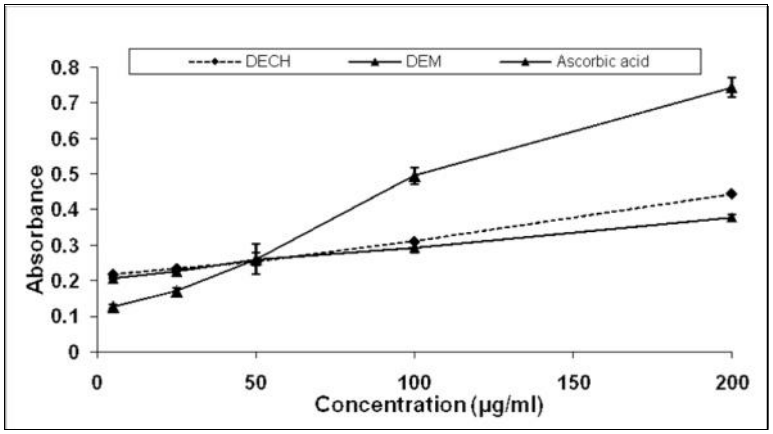

Figure 1
Cupric ion reducing capacity of DECH and DEM extracts of *D. esculentum*.

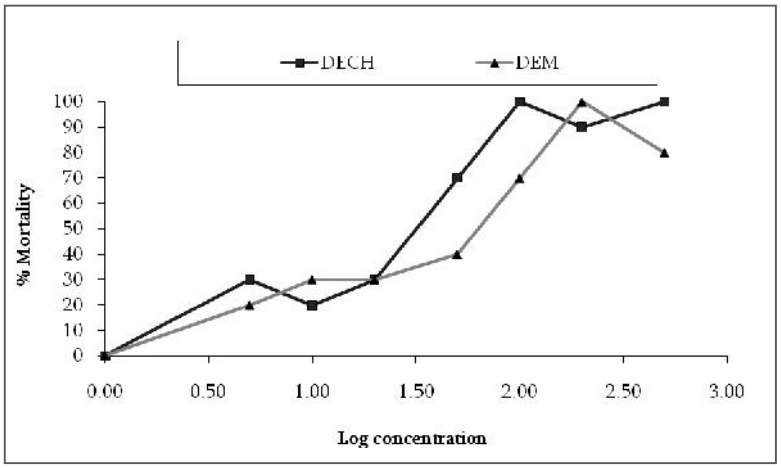

Figure 2
% Mortality of DECH and DEM extracts of *D. esculentum* in Brine shrimp lethality bioassay.

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