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EVALUATION OF ANTIOXIDANT, ANTIMICROBIAL AND THROMBOLYTIC ACTIVITY OF *ELEOCHARIS DULCIS* (CYPERACEAE) FRUITS OF METHANOL EXTRACT

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Abstract

The Chinese waterchestnut (*Eleocharis dulcis*) is the corm of a sedge that grows in water and is commonly used in Asian foods. The edible part consists of starch-rich, non-lignified storage parenchyma interspersed with vascular strands. The present study was carried out to investigate phytochemical, antioxidant, antimicrobial, thrombolytic activity and estimation of total flavonoid, total phenolic, total tannin content of *Eleocharis dulcis* (Cyperaceae) fruits of methanol extract. Total flavonoids content was spectrophotometrically determined using aluminum chloride colorimetric assay while total phenolic and tannin content by Folin Chiocalteu's reagent. Antioxidant activity was determined by DPPH free radical scavenging. Antimicrobial activity was evaluated by disc diffusion assay. The fruit extract contains greater amount of total flavonoid (79.99 mg QE/g dry extract), phenolic content (35.27 mg GAE/g dry extract) and phenolic content (35.27 mg GAE/g dry extract). Fruit extracts showed DPPH scavenging (157.45 µg/mL) that was comparable to standard ascorbic acid (14.15 µg/mL). In disc diffusion assay extract showed greater antibacterial activity. In the thrombolytic activity extracts showed clot lysis for 200 and 100 mg (86.87%) and (84.15%); respectively. It can be revealed that the fruit extract of *Eleocharis dulcis* (Cyperaceae) possess antioxidant and antimicrobial and thrombolytic activity. 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.

Key words: *Eleocharis dulcis, Antioxidant, Antimicrobial, Thrombolytic.*

Introduction

Plants have reliably been utilized by mankind to alleviate and fix the different issues. These days, in different parts of the world customary drug, being replaces by ordinary prescription [1]. *Eleocharis dulcis* is the botanical name of a medicinal plant which is locally known as Pani Faul, Singara and Water Chestnut, belongs to the family of Cyperaceae and it's especially eaten as a fruit in their ripen condition [2]. This plant is grown in natively to eastern Asia and China, Indonesia and Australia [3, 4]. The eatable part of this plant is the underground corm, which is typically harvested. The leaves of this plant are the hollow shape and it would be on an average 1.5 feet in height, and appearance like grassy [5]. The fruit is drop-shaped to broadly drop-shaped as well as it is a perennial herb with short rhizomes [6]. It was especially used for treating jaundice, abdominal mass, conjunctival congestion, throat swelling and pain, excrescence, bloody diarrhoea, hypertension, chronic nephritis, constipation. It has obvious in vivo and in vitro antimicrobial effects on bacteria which are inhibited many various inflammations [7, 8].

Antioxidants are a thing that can prevent or slow damage of cells caused by free radicals, unsteadfast molecules that the body produces as a reaction to environmental as well as other pressures. [9]. Oxygen is a primary component of living. Oxygen arbitrates chemical reactions that metabolize fats, proteins, as well as carbohydrates to create energy [10]. Enormous physiological and biochemical processes in the human body may be produced oxygen-centered free radicals as well as other reactive oxygen species as a by-product. Overproduction of such free radicals is responsible for causing oxidative damage to biomolecules. [11]. Antioxidant-based drug formulations are used for the prevention and treatment of perplexing diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [12]. Phenolic compounds are usually found in both eatable and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Although, other phenolics and flavonoids have been expressed to act as a preventive prelude in the development of cancer and heart disease. [13]. In particular, flavonoids, exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic as well as vasodilatory actions [14].

Thrombolytic therapy is another name of thrombolysis and it is a treatment to disappear dangerous clots in blood vessels, improve blood flow, and prevent damage to tissues and many organs. In general, Thrombolysis is applied as an emergency treatment to vanish blood clots that form in arteries feeding the heart as well as a brain that is the main cause of heart attacks and ischemic strokes [15]. Thrombolysis also may be correlative with an increased risk of complications in patients who are pregnant or at an older age, and in people with other conditions. Patients who sustain thrombolysis have a tiny risk of infection and a slight risk of an allergic reaction to the antithetical dye that may be needed for imaging [16].

Antimicrobial agents act to destroy entire types of microorganisms such as bacteria (antibacterial), viruses (antiviral), fungi (antifungal) and protozoa (antiprotozoal) [17]. Antimicrobial compounds that play an essential role in the natural advocacy of all kinds of living organisms [18]. The antimicrobial compounds from natural sources may be inhibited bacteria by a different mechanism than the currently used antibiotics and may have clinical value in the treatment of resisting microbial strains [19].

Methods

Plant Materials

The edible (fruit) part of the plant of Eleocharis dulcis was collected from near Jahangirnagar University fields, Dhaka, Bangladesh. The plant was identified and authenticated by Bangladesh National Herbarium.

Drying and Grinding

The collected fruits were separated from undesirable materials. Then these were dried in for one week in the sunlight and these were cut into small pieces. The fruit parts were converted into coarse powder by using a suitable grinder. The

powder was kept in a cool, dark and dry place and stored in an airtight container until analysis initiated.

Preparation of Plant Extract

At first, a clean flat flat-bottomed glass container was taken and added about 400 gm of a powdered sample into the container. Then 1500 ml of 90% methanol added into the container and soaked the powder into the methanol. Afterward, the container was sealed with its contents and kept for a period of 10 days accompanying occasional shaking and stirring. After that, the coarse part of the fruits was separated from the mixture by using white cotton. Then the liquid portion was also filtered three times with the help of white cotton. Then again, it was filtered through Whatman filter paper. Then the filtrate was kept in a Rotary evaporator machine that separates the solvent and the desirable crude extract was obtained.

Antimicrobial activity test:

Three pathogenic bacterial strains were used to evaluate antibacterial activity. Two of them were Gram-negative (Klebsiella Oxytoca, Vibrio metschnikovii) and one was gram-positive (Bacillus subtilis). All of the bacterial strains were collected from the Microbiology Lab of the Department of Pharmacy, Dhaka University, Dhaka, Bangladesh.

Phytochemical screening:

Different phytochemical groups such as alkaloids, glycosides, flavonoids, tannins, gums, saponins, steroids were identified by characteristic colour change using standard chemical tests [20]. Molisch Test and Fehling's test were used for carbohydrate existence. Biurets's Test was used for Proteins detection. Flavonoid Test was used for the detection of flavonoids. Alkaloids were detected using the Dragendroff's, Mayer's and Hager's test. For identification of tannin potassium dichromate test, ferric chloride, and lead acetate tests were followed. Keller- Kiliani tests were performed to identify glycosides. Frothing Test for saponins existence, Sulphuric acid test was performed for the detection of steroid. Molisch test was performed for detecting the amount of existence of gum in the samples.

Determination of total flavonoid content

Total flavonoid content was measured by the aluminium chloride quantitative analysis assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water than it was taken in 10 ml of volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg /ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with a UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of the extract [21].

Determination of total phenolic content

The concentration of phenolics in plant extracts was determined using the spectrophotometric method. The total phenol content method was determined by Folin-ciocalteu assy method. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water and it was taken in a volumetric flask (25 ml). One milliliter of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of the extract [22, 23].

Determination of tannin Content

The tannins were determined by the Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of

Folin-Ciocalteu phenol reagent, 1 ml of 35 % Na2CO3 solution and dilute to adding 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. Again, A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with a UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of the extract [24, 25].

DPPH radical scavenging assay

The radical scavenging activity of the extract was quantitatively estimated on the basis of its ability to scavenge the free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH). At first stock solution (1024 µg/mL) of the samples was prepared. From that solution, different concentrations (512–1 µg/mL) of the sample were prepared. In 1 mL of each concentration, 3 mL of 0.1 mM alcoholic DPPH solution was added. After 30 min of incubation in dark at room temperature, absorbance was taken at 517 nm. Ascorbic acid was used as standard. The percentage of DPPH free radical scavenging activity of each extract and standard were calculated as DPPH radical–scavenging activity (1%)

Where, Ao is the absorbance of the control solution containing all reagents except plant extracts, A is the absorbance of the DPPH solution containing plant extract. Finally, the concentration of the sample required to scavenge 50% DPPH free radical (IC50) was calculated from the plot of inhibition (%) against the concentration of the extract [26].

Thrombolytic activity test

The thrombolytic test was carried out by the percentage of the clot lysis method. In short, blood was skimmed from healthy volunteers (n=3) without a history of oral contraceptive or anticoagulant therapy and 1.0 ml of venous blood was transferred to each pre-weighed microcentrifuge tubes and incubated at 37° C for 45 min and was allowed to clot. The thrombolytic activity of all extracts was evaluated using streptokinase (SK) as the standard substance. The extractive (100 mg) from each plant was suspended in 10 ml of distilled water and was kept overnight. Then the soluble supernatant was blended and filtered through a 0.22-micron syringe filter. After clot formation, the serum was completely withdrawn without disturbing the clot and each tube containing the clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube weight of tube alone). To each microcentrifuge tube with the pre-weighed clot, 100 µl aqueous solution of crude extract was added separately. Then, 100 µl of streptokinase (30,000 IU) and 100 µl of distilled water were separately added to the positive and negative control tubes, respectively. All tubes were then incubated at 37° C for 90 min and observed for lysis of the clot. After incubation, the released fluid was removed, and tubes were again weighed to observe the difference in weight after clot disruption [27,28,29].

Test of antimicrobial activity by disc diffusion method

In this method-measured amount of the test, samples are dissolved indefinite volumes of solvent to give solutions of known concentration (µg/ml). Then sterile material filter paper discs are impregnated with a known amount of test substances using micropipette and dried. Standard antibiotic discs and discs on which the solvent used to dissolve the samples are adsorbed and dried are used as a positive and negative control, respectively. These discs are then placed in Petri dishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using a sterile transfer loop for anti-microbial screening. The plates are then kept at 40°C for facilitating maximum diffusion. The test material diffuses from the discs to the surrounding medium. The plates are then kept in an incubator (37° C) for 12-18 hours to allow the growth of the microorganisms. If the test material has any anti-microbial activity, it will inhibit the growth of microorganism giving a clear, distinct zone called "zone of inhibition". The antibacterial activity of the test agent is determined by measuring the diameter of the zone of inhibition in

terms of a millimeter. The experiments are carried out three times and the mean of the reading is recorded [30].

Results

In the phytochemical screening, the extracts revealed the presence of some of the pharmacologically active phytochemicals. After completing a wide range of chemical tests for the identification of major classes of therapeutically important compounds, alkaloid, carbohydrate, Glycoside, tannins, flavonoids, Saponin protein, and diterpenes Triterpenoids.

Total Flavonoid content of the extract was estimated by using the equation and found out to be 1.813±0.433751 QE/g dry extract respectively. In contrast, the absorbance values obtained in the total tannin content test using various concentrations of quercetin were plotted against respective concentrations. A standard calibration curve was obtained with the equation y = 0.3749× - 0.4442 (R2 = 0.9803) (Figure-1)

In the case of total phenolic content, the absorbance values obtained in the test using different concentrations of gallic acid were plotted against respective concentrations. A standard calibration curve was obtained with the equation y = 0.1209x+0.0034 (R2 = 0.5342).The total phenolic content of the methanolic extracts of Eleocharis dulcis was determined using the equation and found to be 0.4266±0.154 GAE/g dry extract respectively. The total tannin content of the extracts was estimated by using the equation and found out to 0.1784±0.065 mg QE/g dry extract respectively(Figure 2).

In the DPPH radical scavenging assay, antioxidant activity was gradually increased with increasing concentration of the extract and the IC50 value was found to be 0.3046±0.024371µg/mL. Also, the absorbance values obtained in the total DPPH content test using different concentrations of quercetin were plotted against respective concentrations. A standard calibration curve was obtained with the equation y = 0.0004× + 0.212 (R2 = 0.8166)(Figure 4) .

The antimicrobial effects of methanol fruit extract against different test organisms are shown in table 1. The extract was showed moderate inhibitory activity against all of these organisms compared with standard ciprofloxacin (Table 1)

The thrombolytic activity was determined by two concentrations of extract as well as compared with two concentrations of standard. The lysis of clot for extract (200mg) & extract (100mg) are 86.87% and 84.15% compared whereas the clot lysis for standard streptokinase (30,000IU) & streptokinase (15,000 IU) are 87.33% & 82.11%.and the lysis by control is quiet negligible 2.22% & 3.30%. $p<0.05$ was considered statistically significant & the result of significance is $p< 0.00002$. Since the extract solution showed enough clot lysis compared with the standard. As a result, the extract has a thrombolytic activity (Table 2)

Discussion

The sources plants offer as a wellspring of well available, conservative and equipped prescription from ancient times. Phenolic and flavonoid mixes are thought very crucial auxiliary metabolites for natural exercises [31].

Phenolic mixes have remedial potential against different sorts of illnesses in view of their cell reinforcement properties. Flavonoids are a gathering of polyphenolic substances present in many plants and are in charge of various biochemical and antimicrobial exercises. Cancer prevention agents and antimicrobial properties of flavonoids from plant extract announced in various investigations [32, 33]. It advertises the antioxidant activity via radical scavenging, metal ion chelation, and membrane protective efficacy. Consequently, plant segments like tannins can be in charge of cell reinforcement property [34]. DPPH test has been utilized as a brisk, dependable, just as a reproducible parameter for screening in vitro cell reinforcement movement of plant removes. It tends to be additionally used to measure the gift capacity of electron of regular items [35].

In an antibacterial measure, the methanol unrefined extract was found to have low inhibitory properties against exploratory pathogenic microbes' species. The results obtained in the

present study revealed that Gram-positive bacteria were more sensitive than Gram-negative bacteria to the tested extract. The difference of the inhibition zone diameter depends primarily upon many factors namely the diffusion capacity of substances (present in the extracts) in the agar medium, antibacterial activity of diffused substances, growth and metabolic activity of bacteria in the medium. Inhibition zone diameter can further be associated with polarities of substances which make up the tested extracts and also with cell wall composition of test organisms since Gram-positive bacteria present cell walls with lower lipid levels than do Gram-negative bacteria. Eleocharis dulcis fruit extract reflects good antibacterial activity against the bacterial strains Vibrio metschnikovii, Bacillus subtilis and Klebsiella Oxytoca has medium activity against Bacillus subtilis and Klebsiella Oxytoca. Standard antibiotic discs of Ciprofloxacin were used for comparison purposes [36, 37, and 38].

Platelets assume a fundamental job during the time spent development of thrombus by sticking to be harmed areas of the endothelial surface. The enacted platelets structure platelets to platelets bonds and separated from tie to the leucocytes and bring off them into a baffling strategy for plaque arrangement just as the development [39]. Streptokinase shapes a 1:1 stochiometric complex with plasminogen which is capable of changing over extra plasminogen to plasmin [40]. The obtained results suggest its further advanced investigation.

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References

1. Rebaya A, Belghith SI, Baghdikian B, Leddet VM, Mabrouki F, Olivier E, Cherif JK, Ayadi MT. Total phenolic, total flavonoid, tannin content, and antioxidant capacity of Halimium halimifolium (Cistaceae). Journal of applied pharmaceutical science. 2014;5(1):52-7.

2. Favas, Paulo JC, et al. "Accumulation of uranium by aquatic plants in field conditions: prospects for phytoremediation." Science of the total environment 470 (2014): 993-1002.

3. Xiao, Luyao, et al. "Structural and physicochemical properties of chemically modified Chinese water chestnut [Eleocharis dulcis (Burm. f.) Trin. Ex Hensch] starches." International journal of biological macromolecules 120 (2018): 547-556.

4. Ghazanfar SA. Eleocharis dulcis (kuta), a plant of economic and cultural importance in the South West Pacific: habitat restoration efforts in the vanua of Buca, Vanua Levu, Fiji. The South Pacific Journal of Natural and Applied Sciences. 2001; 19(1):51-3.

5. Water chestnut (Eleocharis dulcis). Available from https://growerjim.blogspot.com/2016/01/water-chestnut-eleoch Water chestnut (Eleocharis dulcis). Accessed 12th February 2019.

6. NATIONAL PARKS. Eleocharis dulcis (Burm.f.) Trin. Ex Hensch. Available from https://florafaunaweb.nparks.gov.sg/special-pages/plant-detail.aspx?id=5462. Accessed 12th February 2019.

7. Liu HB, You YN, Zhu ZX, Zheng XF, Huang JB, Hu ZL, Diao Y. Leaf transcriptome analysis and development of SSR markers in water chestnut (Eleocharis dulcis). Genet. Mol. Res. 2015 Jan 1; 14:8314-25.

8. You Y, Duan X, Wei X, Su X, Zhao M, Sun J, Ruenroengklin N, Jiang Y. Identificationof Major Phenolic Compounds of Chinese Water Chestnut and their Antioxidant Activity. Molecules. 2007 Apr 25; 12(4):842-52.

9. Zhan, Ge, et al. "Study on antibacterial properties and major bioactive constituents of Chinese water chestnut (Eleocharis dulcis) peels extracts/fractions." European Food Research and Technology 238.5 (2014): 789-796.

10. Nie, Hui, et al. "Identification of compounds from chufa (Eleocharis dulcis) peels with inhibitory acrylamide formation activity." Revista Brasileira de Farmacognosia (2018).

11. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life sciences. 2004 Mar 12; 74(17):2157-84.

12. Khalaf NA, Shakya AK, Al-Othman A, El-Agbar Z, Farah H. Antioxidant activity of some common plants. Turkish Journal of Biology. 2008 Feb 19; 32(1):51-5.

13. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. Journal of agricultural and food chemistry. 1999 Oct 18; 47(10):3954-62.

14. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of agricultural and food chemistry. 1998 Oct 19; 46(10):4113-7.

15. Aasim, Muhammad, et al. "Multiple Uses of Some Important Aquatic and Semiaquatic Medicinal Plants." Plant and Human Health, Volume 2. Springer, Cham, 2019. 541-577.

16. Dhiman, Nisha, Vanita Patial, and Amita Bhattacharya. "The Current Status and Future Applications of Hairy Root Cultures." Biotechnological Approaches for Medicinal and Aromatic Plants. Springer, Singapore, 2018. 87-155.

17. Aizawa, Tomoko, et al. "Burkholderia heleia sp. nov., a nitrogen-fixing bacterium isolated from an aquatic plant, Eleocharis dulcis, that grows in highly acidic swamps in actual acid sulfate soil areas of Vietnam." International journal of systematic and evolutionary microbiology 60.5 (2010): 1152-1157.

18. Rauha JP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International journal of food microbiology. 2000 May 25; 56(1):3-12.

19. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of ethnopharmacology. 1998 Feb 1; 60(1):1-8.

20. Al-Daihan S, Al-Faham M, Al-shawi N, Almayman R, Brnawi A, shafi Bhat R. Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. Journal of King Saud University-Science. 2013 Apr 1; 25(2):115-20.

21. Har L, Intan SI. Antioxidant activity, total phenolics and total flavonoids of Syzygium polyanthum (Wight) Walp leaves. International Journal of Medicinal and Aromatic Plants. 2012; 2(2):219-28.

22. Ghasemzadeh A, Jaafar HZ, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). Molecules. 2010; 15(6):4324-33.

23. Stankovic MS. Total phenolic content, flavonoid concentration and antioxidant activity of Marrubium peregrinum L. extracts. Kragujevac J Sci. 2011 Jan 1; 33(2011):63-72.

24. Singh R, Verma PK, Singh G. Total phenolic, flavonoids and tannin contents in different extracts of Artemisia absinthium. Journal of Complementary Medicine Research. 2012; 1(2):101-4.

25. Afify AE, El-Beltagi HS, El-Salam SM, Omran AA. Biochemical changes in phenols, flavonoids, tannins, vitamin E, β–carotene and antioxidant activity during soaking of three white sorghum varieties. Asian Pacific Journal of Tropical Biomedicine. 2012 Mar 1; 2(3):203-9.

26. Anisuzzman M, Zilani MN, Khushi SS, Asaduzzman M. Antioxidant, antibacterial potential and HPLC analysis of Dioscorea alata bulb. Indonesian Journal of Pharmacy. 2016 Jan 1; 27(1):9.

27. Azad AK, Lasker S, Irfan-Ur-Rahaman OI, Sharmin Akter M, Khairuzzaman MI, Sultana C, Das N, Ahmeda N, Hossain S, Haque S, Biswas K. Comparative studies on antioxidant and thrombolytic activities of methanol and ethylacetate extracts of trema orientalis.

28. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Effect of Fagonia arabica (Dhamasa) on in vitro thrombolysis. BMC

Complementary and Alternative Medicine. 2007 Dec;7(1):36.

29. Ramjan A, Hossain M, Runa JF, Md H, Mahmodul I. Evaluation of thrombolytic potential of three medicinal plants available in Bangladesh, as a potent source of thrombolytic compounds. Avicenna journal of phytomedicine. 2014 Nov; 4(6):430.

30. Wiart C, Mogana S, Khalifah S, Mahan M, Ismail S, Buckle M, Narayana AK, Sulaiman M. Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. fitoterapia. 2004 Jan 1; 75(1):68-73.

31. Arias JP, Zapata K, Rojano BI, Penuela M, Arias M. Plant cell suspension culture of Thevetia peruviana (Pers.) K. Schum. In shake flask and stirred tank reactor scale: A comparative study. African Journal of Biotechnology. 2017 Dec 27; 16(52):2355-63.

32. Lin Y, Shi R, Wang X, Shen HM. Luteolin, a flavonoid with potential for cancer prevention and therapy. Current cancer drug targets. 2008 Nov 1; 8(7):634-46.

33. López-Lázaro M. Distribution and biological activities of the flavonoid luteolin. Mini reviews in medicinal chemistry. 2009 Jan 1; 9(1):31-59.

34. Zhang LL, Lin YM. Tannins from Canarium album with potent antioxidant activity. Journal of Zhejiang University Science B. 2008 May 1; 9(5):407-15.

35. Gul MZ, Ahmad F, Kondapi AK, Qureshi IA, Ghazi IA. Antioxidant and antiproliferative activities of Abrus precatorius leaf extracts-an in vitro study. BMC complementary and alternative medicine. 2013 Dec; 13(1):53.

36. Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. Journal of advanced pharmaceutical technology & research. 2011 Apr; 2(2):104.

37. Zilani MN, Sultana NA, Bakshi MK, Shampa IJ, Sumi SJ, Islam O. Bioactivities of leaf and root extract of Ceriscoids turgida (Roxb.). Oriental Pharmacy and Experimental Medicine. 2018 Feb 21:1-7.

38. Moshi MJ, Mbwambo ZH. Some pharmacological properties of extracts of Terminalia sericea roots. Journal of Ethnopharmacology. 2005 Feb 10; 97(1):43-7.

39. Dewan SM, Das A. Investigation of in vitro thrombolytic potential and phytochemical nature of Crinum latifolium L. leaves growing in coastal region of Bangladesh. Int J Bio Pharm Res. 2013; 4(1):1-7.

40. Banerjee A, Chisti Y, Banerjee UC. Streptokinase—a clinically useful thrombolytic agent. Biotechnology advances. 2004 Feb 1; 22(4):287-307.

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Table 1: In vitro antibacterial activity of *Eleocharis dulcis*.

Bacterial Strains	Type of bacteria	Diameter of zone of inhibition			
		Blank	Ciprofloxacin (50µg/disc)	Extract (250 µg/disc)	Extract (500 µg/disc)
Klebsiella Oxytoca	Gram(-)	-	40mm	16mm	7mm
Bacillus subtilis	Gram(+)	-	40mm	14mm	21mm
Vibrio metschnikovii	Gram(-)	-	45mm	29mm	19mm

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

Table 2: % of clot lysis in different concentrations of standard, Sample and control

Treatment	Concentrations	% of cot lysis
Extract	200mg	86.87
	100mg	84.15
Standard	30,000IU	87.33
	15,000IU	82.11
Control	-	2.22
	-	3.30

Figure 1: Total flavonoid content of *Eleocharis dulcis.*

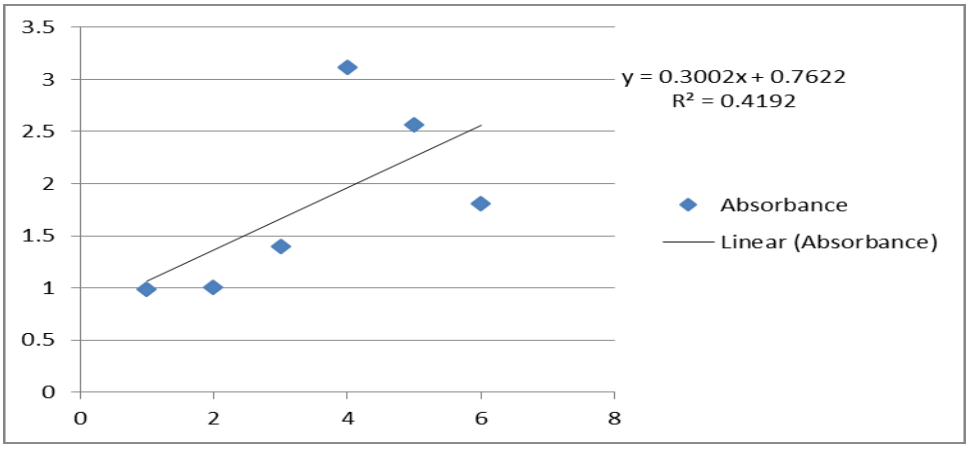


Figure 2: Total phenolic content of *Eleocharis dulcis*.

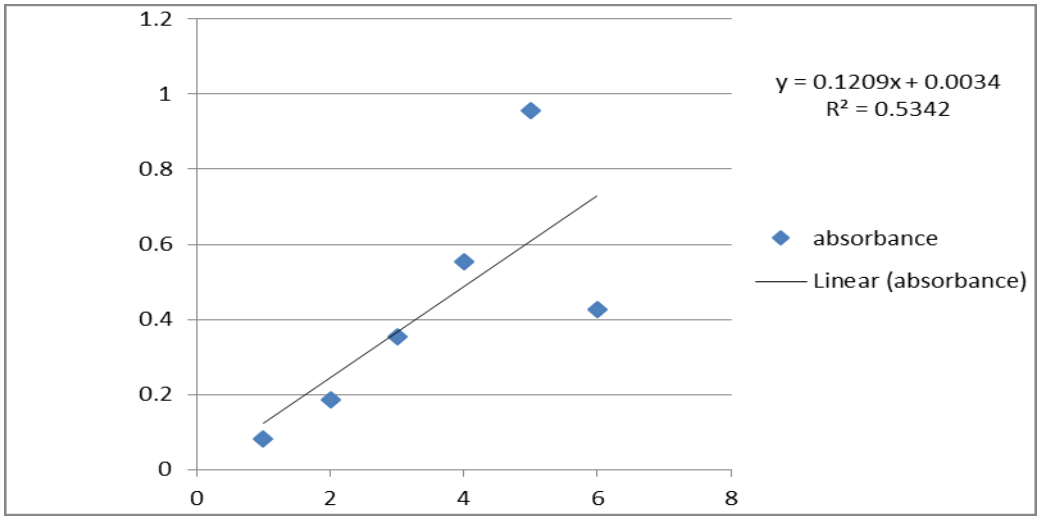


Figure 3: Total tannin content of *Eleocharis dulcis*.

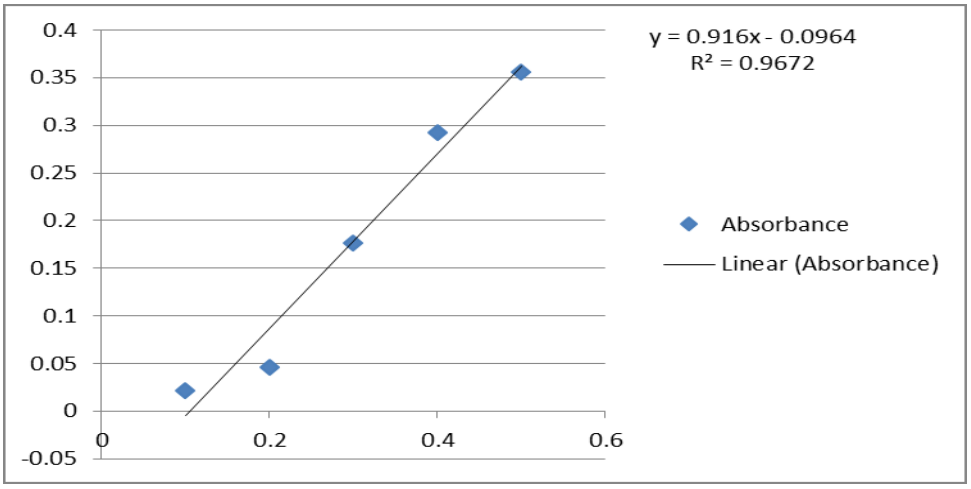
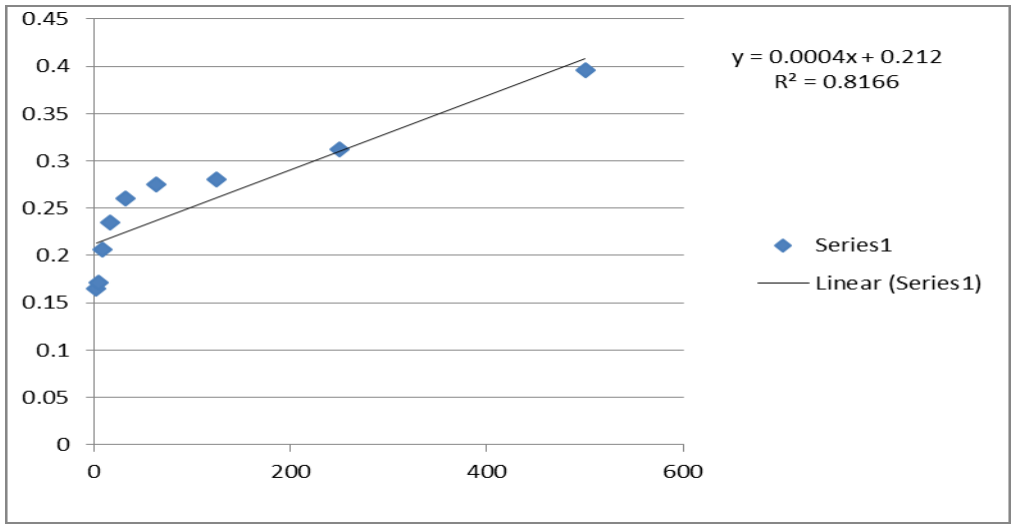


Figure 4: Total DPPH content of *Eleocharis dulcis*.



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