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Qualitative, Quantitative Analysis Of Bio-Active Phyto-Chemicals And Bio-Assay of *Amaranthus Gangedicus* Seeds.

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ABSTRACT

Phytochemical analysis and bio-activities such as antioxidant, anti-inflammatory of Amaranthus Gangedus seeds has been investigated. The presence of phyto chemicals in the plant was identified by qualitative analysis tests. The antioxidant activity was estimated using DPPH radical scavenging technique and total phenolic contents. The anti-inflammatory activity was estimated by spectrophotometric method.
Keywords: Qualitative analysis, Anti-oxidant, Anti-inflammatory, Spectro photometry

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INTRODUCTION

The bio-active plant products are important for medicinal, pharmaceutical industries and living systems. These products are existing in all parts of the plants such as roots, stems, and leaves, flowers, seeds and seeds coats[1]. Plants have various biologically active compounds such as phenolic, alkaloids, terpenoids, lectins, flavone derivatives, tannins, resin and lactones. These active ingredient products were identified by chemists through qualitative analysis and estimated by quantitative analysis using volumetric and spectroscopic techniques [2-8]. Also the biological activities such as antibacterial, antifungal, antioxidant and anti-inflammatory etc. of the plant protection chemicals have been evaluated. The antibacterial activity of Amaranthus viridius was evaluated by Malik et. al. using gram positive and gram-negative bacterial strains[9]. Rashid and his co-workers[10] evaluated the antimicrobial assay of Amaranthus Viridis(Chowlai) and Cannabis sativa (Bhang) against clinically important microbes. Thrun et. al., investigated the phytochemical, pharmacognostic, antimicrobial and antioxidant activity assay of different extracts of Amaranthus tricolor[11]. Aditya and Bhattacharji [12] have studied the anti-diabetic and antioxidant assay of Amaranthus hypochondriacusL. using GC-MS technique. Salvamani et. al. have to be found that curing of hyper cholesteromia disease with the extract of Amaranthus Viridis and evaluate the anti-HMG-CoA reductase, antioxidant, and anti-inflammatory activities[13]. The antimicrobial, anticancer and antioxidant potential of two vegetable species of Amaranthus in Bangladesh [14] have been reported by Al-Mamun group of researchers. Antioxidant and anticoagulant activities of aqueous leaf extract of Amaranthus Gangeticus L.was studied by Anitha and Ponbavani[15]. The study of phytochemical composition and anti-oxidant assay of two Amaranthus Sp. harvested inCameroons were reported by Amaud Suffo and his co-workers[16].Clemente and Desai [17] have investigated the hypoglycemic, hematological, hypolipidemic and Antioxidant assay of Amaranthus Tricolor Leaf extract. Phytochemical Scientists[18-21] evaluated the antioxidant activity assay of Amaranthus Spinosus Linn. by non-enzymatic haemoglycosylation, Amaranthus Cruentusgrains, Amaranthus Blitum by free radical scavenging activity. The hepatoprotective potential of Amaranthus Hypochondriacus seed was reported by Akin-Idowu et al.,[22].Islam et al. have estimated the quantity of sugar, phenolicand antioxidant assay of Amaranthaceae Accession[23]. Amaranthus Gangedicus is one of the important and conservative remedial plant and it has been utilized to treatment of numerous diseases. It consists of 60-75 species, out of 40 which were in native of USA. This well growed in temperature and tropical area and used as grain or vegetable. It had minerals and vitamins. It cures anti-snake venom, diuric, laxatives, antipyretic etc. diseases. Also it had important bio-potentials such as anthelmintic, anti-androgenic and anti-inflammatory. Literature survey shows no report on the study of qualitative analysis, estimation of antioxidant andanti-inflammatory potential of Amaranthus Gangedicus seeds in present and past. Since, the author reports the analysis of phytochemical composition, assay of antioxidant andanti-inflammatory potential of Amaranthus Gangedicus seeds by making the extract with suitable solvents.

MATERIALS AND METHODS

Materials

The materials used in this investigation are Amaranthus Gangedicus seeds collected from November and December months of the year. All chemical employed in this investigation are AnalaR grade and supplied from E-Merck and Sigma-Aldrich chemical companies. Borosil grade glass-wares used for qualitative analysis. The spectral grade solvents utilized for measurement of absorbance maximum of the samples in Shimadzu UV-3600 UV-visible-NIR spectrometer.

Methods

Preparation of Amaranthus Gangedicus extract

About 20 g of Amaranthus Gangedicus seed powder was dissolved in 200 mL of methanol in Soxhlet apparatus at 64^{o}C. After the extraction process was over it will be dried using anhydrous sodium sulphate. The dried extract was used to systematic phytochemical qualitative analysis to the determination of phenolic, alkaloids, terpenoids, lectins, flavone derivatives, tannins, resin and lactone contents.

Phytochemical analysis [2-8]

Test for phenol, tannin and flavonoid contents (Ferric chloride test)

Freshly prepared 2 mL of 2% ferric chloride reagent was added to 2 mL of the extract. Blue-green or block coloration showed the extract have phenols, tannin and flavones.

Test for alkaloids (Dragendroff's test)

Exactly 2 ml of the extract was mixed with hydrochloric acid, separated the acid layer then it was mixed with 3 drops of freshly prepared Dragendroff's reagent. Yellowish red coloration denotes the alkaloids presence.

Test for carbohydrate (Molisches test)

Exactly 2 ml of Molisches reagents was added to 2 ml of the prepared extract and shaken well. To this 2 mL of concentrated sulphuric acid was added via inside of the tube.

Test for Protein and amino acids (Million's test)

Exactly 2 mL of Million's reagent was mixed with 2 mL extract. the formed white precipitate was turned to red colour by heating gently.

Test for Glycosides (Liebermann's test)

In an ice-cold condition, exactly 2 mL of chloroform and acetic acid were added to 2 mL of the extract. About 2 drops of concentrated sulphuric acid was added to the above reaction mixture. Violet coloration denotes glycosides present.

Test for steroids

About 10 mL of chloroform followed by 5 mL of concentrated sulphuric acid were added to 1 mL of the extract. The red coloration occurs in upper layerand sulphuric acid layer turned yellowish green fluorescence formation denotes steroid present.

Test for Terpenoids (Salkowski test)

Four drops of concentrated sulphuric acid and 2 mL of chloroform were added to 2mL of the extract and allowed to stand for few minutes. Appearance of golden yellow colour indicates the triterpenes present.

Test for Saponin (Froth test)

Exactly 4 mL of the seed extract was mixed with 4 mL offreshly prepared sodium bicarbonate, vigorously stirred the reaction mixture and standwithout disturbance for 3 minutes. The occurrence of honey comb shows the saponin present.

Test for Resins

Exactly 3 ml of extract was dissolved with 3 mL of dimethyl ketone and poured the reaction mixture into 10 mL of distilled water in a beaker. The turbidity formation of shows the resin present.

Estimation of total phenolic contents

The total phenolic assay was estimated by the procedure reported in literature[24, 25].In a series of series of test tubes exact quantity of diluted extract,0.5 mL of distilled water and 0.125 mL of Folin–Ciocalteu reagent were added. The reaction mixture was shaken well and kept aside for 6 min without disturbance, before adding1.25 mL of 7% Na_2CO_3. The total volume of the solution was then adjusted to the final volume of

3 mL with distilled water and well shaken. These tubes were incubated for 30 minutesin the darkness and measured the absorbance at 760 nm was read versus a prepared blank. The total phenol content of seed extract was calculated in the percentage.

Antioxidant bio-assay

Measurement of antioxidant assay by DPPH radical scavenging technique

The DPPH radical scavenging potential assay of Amaranthus Gangedicus seed was estimated by literature method[26-28]. In a fresh tube 3 mL of methanol taken and it is used as a blank. In a second tube added 1 mL of methanol and 0.1mnolar DPPH solution and shake well and it is used as a control, the remaining tubes were filled appropriate volume of the extract and adjusted to 1 mL with methanol and finally added 3 mL of 0.1mmol DPPH and vortexed. All the tubes were incubated for 30 minutes at room temperature. Measured the absorbance of all tubes of the extract. The DPPH radical scavenging activity assay was expressed in terms of

$$\%\ \text{of inhibitioon} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Estimation of hydrogen peroxide activity

The hydrogen peroxide scavenging assay was measured using literature method[29]. The phosphatic buffer solution at the pH of 7 of hydrogen peroxide was prepared with 40 mmol of hydrogen peroxide. The phosphatic buffer with hydrogen peroxide solution was added to 100 µL of extract in 3 mL of distilled water and mixed well. After 10 minutes measured the absorbance at 250 nm of the test sample and blank. The hydrogen peroxide scavenging activity assay was calculated as follows.

$$\%\ \text{of hydrogen peroxide scavenging} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Estimation of anti-inflammatory activity assay

The anti-inflammatory bio-assay of the extract was carried out by the procedure published in literature[30]. Exactly 1.5 mL of bovine serum was added to 0.5 g of solution having 100-500µg different concentration of extract. This reaction mixture was incubated at 27±1 ºC for 20 minutes. At 60 ºC within the period of 15 minutes the denaturation was induced. After cooled to room temperature, measured the absorbance of the turbidity spectrophotometrically at 660nm. By following equation, the inhibition of denaturation of sample and control was calculated.

$$\%\ \text{of antiinflammatory} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

The qualitative analysis of the phytochemical test of Amaranthus Gangedicus was done and the results are tabulated in Table 1. From the qualitative test this plant seed has the phytochemical constituents.

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Table 1:Phytochemical constituent analysis of Amaranthus Gangedicus seed.

Sl. No.	Phytochemical Test	Observation	Inference
1	Ferric chloride test	No blue-green or black colour	Absence of tannin
2	Shinoda test	No change of red to pink colouration	Absence of flavonoids
3	Dragendroff's test	No reddish-brown precipitate obtained	Absence of alkaloids.
4	Molisches test	Violet ring formed	Presence of carbohydrates
5	Millions' test	The white precipitate was turned to red	Presence of protein
6	Salkowski test	Formation of golden yellowish colour	Presence of terpenes.
7	Steroid	Yellowish green fluorescence	Presence of steroids
8	Forth test	No honey comb like forth formed	Absence of saponin
9	Liebermann's test	No violet colour turned to green	Absence of glycosides
10	Resins	Formation of turbidity occur	Presence of Resin

From the qualitative analysis experiments, the Amaranthus Gangedicus seed extract has carbohydrates, proteins, terpenes, steroids and resins phytochemicals.

The large diverse group phenolic compounds containing varieties of aromatic family of plants secondary metabolites. They are the secondary metabolites origin of plants and are classified into non-soluble category such as phenolic acids, tannins, cinnamic acids, quinines flavonoids and lignin's. These are important for plant fertility and reproduction and in many defense reactions to protect abiotic and biotic stress. In this analysis, the obtained whole phenolic contents in the extract was 28%. The absorbance of observed phenolic contents of the seed extract was given in Table 2.

Table 2: The observed total phenolic content of Amaranthus Gangedicus seed extract.

Sl. No.	Sample	Absorbance (OD)
1	T_1	0.5763
2	T_2	1.110
3	T_3	1.983
4	T_4	2.800
5	T_5	2.811
6	E_1	0.833
7	E_2	0.873
Total phenolic content of the extract: 27.65%		

The observed DPPH radical scavenging activity assay analysis was given in Table 3. This value implies the Amaranthus Gangedicus seed extract have considerable quantity of antioxidant activity.

Table 3: The DPPH radical scavenging activity assay of Amaranthus Gangedicus seed extract

Sl. No.	Sample	Absorbance (OD)
1	T_1	0.507
2	C	0.203
Scavenging activity: 65.02%		

The observed hydrogen peroxide scavenging activity of Amaranthus Gangedicus seed extract was presented in Table 4. From the observed values, the extract possess considerable quantity of hydrogen peroxide scavenging assay.

Table 4: The hydrogen peroxide scavenging activity of Amaranthus Gangedicus seed extract

Sl. No.	Sample	Absorbance (OD)
1	T_1	1.134
2	C	1.320
Scavenging activity: 33.04%		

The obtained anti-inflammatory assay of the Amaranthus Gangedicus seed extract was presented in Table 5. This experiment was conducted with various volumes with concurrent concentrations. From the experimental results the author concluded that Amaranthus Gangedicus seed extract have considerable anti-inflammatory assay.

Table 5: Anti-inflammatory bio-assay of Amaranthus Gangedicus seed extract

Sl. No.	Test quantity of extract (mL)	Test concentration of extract	Activity in %
1	0.2	8.5	61.23
2	0.2	17.0	28.04
3	0.3	26.5	19.02
4	0.4	35.0	11.23
5	0.5	42.5	8.01
Minimum volume of extract requirement is 0.15 mL			
The IC_{50} of inhibition concentration is 13.35 mg			
% of minimum inhibition is 25.03			

CONCLUSIONS

The good wealthy Amaranthus Gangedicus seeds collected and prepared the extract with methanol. This extract was analyzed for finding the phytochemical contents through qualitative analysis. Based on the qualitative analysis, the extract possesses carbohydrate, protein, steroid, resin and terpenes. The bio-assay of antioxidant and anti-inflammatory of this extract was determined and the experiment results shows significant antioxidant and anti-inflammatory assays.

REFERENCES

[1] Khatun, A., Rahman, M., Jahan, S. Preliminary phytochemical and pharmacological screening of Murraya exotica Linn. leaves extract. Orient. Pharm. Exp. Med., 2014, 14 223–229.

[2] Aboubacar, A., Hamaker, B. R. Low molecular weight soluble starch and its relationship with sorghum couscous stickiness. J. Cereal Sci. 2000, 31:119-126.

[3] Walsh, E. O. An Introduction to Biochemistry. London: The English Universities Press Ltd. 1961, 406–407. 1961

[4] Nayeem, A. A., Khatun, A., Rahman, M. S., Rahman, M. Evaluation of phytochemical and pharmacological properties of Mikania cordata (Asteraceae) leaves. J. Pharm. Phytother., 2011, **3** (8): 118–123.

[5] Ghani, A. Medicinal Plants of Bangladesh (1st ed.). Dhaka, Bangladesh: The Asiatic Society of Bangladesh., 1998, 505.

[6] Langenhein, J. Plant Resins: Chemistry, evolution, ecology, and ethnobotany, Timber Press, Portland, OR. 2003.

[7] Grimaldi, A. D., American Museum of Natural History Window to the Past, 1996, 16-20,

[8] Fiebach, K., Grimm, D. Resins, Natural". Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley-VCH. 2000. doi:10.1002/14356007.a23-073.

[9] Malik, K., Farkhanda N., Nisar. N. Antibacterial Activity of Amaranthus Viridis.Bull. Env. Pharmacol. Life Sci., 2016, 5, 76-80.

[10] Rashid, F., Butt, F. A., Nasreen, S., Nisa, F. U., Kanwal, Z., Kaleem, A., Andleeb, A. S. In Vitro Antimicrobial and Antioxidant Activities of Two Medicinal Plants Against Some Clinically Important Bacteria., Fuuast J. Biol., 2016, 6(1), 103-107.

[11] Tharun, Nagavenkateshwar Rao, K., Krushna Padhy, S., Dinakaran, S. K., Banji, D., Avasarala, H., Ghosh, S., Prasad, M. S. Pharmacognostic, Phytochemical, Antimicrobial and Antioxidant Activity Evaluation of Amaranthus tricolor Linn. Leaf**.** Asian J. Chem., 2012, 24, 455-460.

[12] Aditya, M., Bhattacharjee. S. Foliar anti-diabetic and antioxidant potential of a promising accession of Amaranthus hypochondriacus L.: GC-MS based evidences. The J. Phytopharmacol., 2018, 7(2),121-126.

[13] Salvamani, S., Gunasekaran, B., Shukor, M. Y., Shaharuddin,N. A., Sabullah, M. K., Ahmad, S. A. Anti-HMG-CoA Reductase, Antioxidant, and Anti-Inflammatory Activities of Amaranthus viridisLeaf Extract as a Potential Treatment for Hypercholesterolemia. Evidence-Based Comp. Alt. Med., 2016, Article ID 8090841, 10 pages, http://dx.doi.org/10.1155/2016/8090841.

[14] Al-Mamun, M. A., Husna, J., Khatun, M., Hasan, R., Kamruzzaman, M., Hoque, K. M. F., M. Abu Reza, M., Ferdousi, Z. Assessment of antioxidant, anticancer and antimicrobial activity of two vegetable species of Amaranthus in Bangladesh. BMC Complemen. Alt. Med., 2016, 16:157. DOI 10.1186/s12906-016-1130-0.

[15] Anitha. R, M., Ponbavani, S. Antioxidant and anticoagulant activity in Amaranthus gangeticus l. Aqueous leaf extract, World J. Pharm. Pharm. Sci., 2011, 2, 2682-2688.

[16] Arnaud Suffo, K. L., Ashish, R., Tedonkeng, P., Kuiate, J. R. Effect of Processing Methods on Chemical Composition and Antioxidant Activities of Two Amaranthus Sp. Harvested in West Region of Cameroons. J.Nutr. Food Sci., 2016, 6:2. DOI: 10.4172/2155-9600.1000477

[17] Clemente, A. Ce., Desai, P. V. Evaluation of the Hematological, Hypoglycemic, Hypolipidemic and Antioxidant Properties of Amaranthus Tricolor Leaf Extract in Rat. Trop. J. Pharm. Res., 2011, 10 (5), 595-602.

[18] Baral, M, Biswas, S., Chakraborty, S., Ghosh, A. K., Teixeira Da Silva, J. A., Panda, S., Chakraborty, P. In Vitro Antioxidant Activity of The Whole Plant of Amaranthus Spinosus Linn. Int. J. Biomed. Pharm. Sci., 2011, 5 (1), 75-78.

[19] Cherian, P., Sheela, D. Antioxidant Activity of Ethyl Acetate Extract of Genus Amaranthus Linn. Int. J. Pharm. Sci. Res., 2017, 28. 5199-5204

[20] Ashok Kumar, B. S., Lakshman, K., Jayaveera, K. N., Saleemulla K, Manoj, B., Narayan Swamy, V.B. Evaluation of The Antioxidant Activity of Amaranthus Spinosus Linn. By Non-Enzymatic Haemoglycosylation. Sains Malaysiana.,2010, 39(3), 413–415

[21] Paśko, P., Bartoń, H., Fołta, M., Gwiżdż, G. Evaluation of antioxidant activity of amaranth (Amaranthus Cruentus) Grain and By-Products (Flour, Popping, Cereal). Roczn. Pzh., 2007, 58, 35-40.

[22] Akin-Idowu, P. E., Odunola, O. A., Gbadegesin, M. A., Aduloju, A. O., Owumi, S. A., Adegoke, A. M. Hepatoprotective effect of Amaranthushypochondriacusseed extract on sodium arseniteinduced toxicity in male Wistar rats. J. Med. Plant Res., 2015, 9(26),731-740.

[23] Islam, S., Akond, M., Islam, I., Adam, Z. Soluble sugar contents, total phenolic, and antioxidant capacity in a diverse set of amaranthaceae accessions. Int. Food Res. J., 2017, 24(5), 2009-2016.

[24] Medini, F., Fellah, H., Ksouri, R., Abdelly, C.Total phenolic, flavonoid and tannin contents and antioxidant andantimicrobial activities of organic extracts of shoots of the plant Limonium delicatulum. J. Taibah Univ. Sci. 2014, 8, 216–224.

[25] Dewanto, X., Wu, K., Adom, K., Liu, R.H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity, J. Agric. Food Chem. 2002, 50, 3010–3014.

[26] Saranya P., Gurusamy, K. Free Radical Scavenging Activity of Amara_ThusBlitum. Pharmacologyonline,2010,3, 625-636.

[27] Thirunarayanan, G. Green approach using water mediated Diels-Alder reaction for the synthesis of some 2-(4-bromo-1-naphthyl)-3-(aryl) bicyclo [2.2.1] hept-5-ene methanones and evaluation of their antimicrobial and antioxidant activities, MacidonianJ. Chem. Chem. Engg., 2007, 36, 1-14.

[28] Thirunarayanan, G.,Vanangamudi, G., Synthesis, spectral studies, antimicrobial, antioxidant and insect antifeedant activities of some 9H-fluorene-2-yl keto-oxiranes, Spectrochim. Acta, 2011, 81A, 390-396.

[29] Khana, U. K. S., Oba, S. Bioactive Substances in Leaves of Two Amaranth Species, Amaranthus Tricolor and A. Hypochondriacus. Can. J. Plant Sci., 2013, 93, 47-58.

[30] Lipkin, A., Veronika, A., Aleksandra, N., Alekse, B., Eberhardt, K., Mikhae, L.B., Eugene, G., Tsezi, E., An Antimicrobial Peptide Ar-AMP from Amaranth (Amaranthus RetroflexusL.) Seeds. J. Sci. Direct., 2004, 34, 93-95.