



Preliminary Phytochemical Screening of *Amaranthus tricolor* (Linn) Leaves in various solvents.

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ABSTRACT:

The genus *Amaranthus* has potential activity as a medicinal herb. The present phytochemical investigation focuses on evaluation of the efficacy of ethyl acetate, chloroform, n-hexane, acetone extract of leaves of *Amaranthus tricolor* Linn. The leaves were collected from the India. The all four extract of leaves of *A. tricolor* Linn. was prepared and phytochemical screening was done. The extract was prepared by Soxhlet extraction method. The test tube reactions shows and examination revealed the presence of phenols, alkaloids, glycosides, proteins and flavonoids in chloroform, ethyl acetate, n-hexane, acetone and extracts of *A. tricolor*. By present work, it can be concluded that the plant *A. tricolor* is endowed with a significant medicinal activity due to the presence of active constituents like polyphenolic contents and, thus justifying its use in the indigenous system of medicine. The results of primary phytochemical screening, preliminary phytochemical tests were satisfactory and can be correlated with earlier reports.

Keywords: *Amaranthus tricolor*, n-hexane, ethyl acetate, chloroform.

INTRODUCTION:

Amaranthus tricolor L. (Amaranthaceae) is an ornamental plant known as Joseph's coat in English and 'laal shak' in the local (Bengali) language. The plant is both cultivated in Bangladesh, and can be found growing on fallow lands. *Amaranthus* are considered as dominant leafy vegetables in temperate and tropical regions 1,2,3. *Amaranthaceae* is a family of 70 species where 4 species are cultivated as leafy vegetables in this region. *Amaranthus tricolor* Linn is one of the most extensively consumed vegetables in Bangladesh due to its attractive color, nutritious value, and delicious flavor 4. The fundamental knowledge about the characteristics of the leaves, seeds, and flour is crucial for the promotion of the crop for use in the food industry. The *Amaranthus tricolor* L.(cv. Valentina) leaf extracts do not only have beautiful crimson color, they also contain a large number of biologically active substances and can be used for tea drinks preparation 5. In spite of tremendous efforts made in the field of modern medicine, there is hardly any drug that stimulates liver function, offer protection to the liver from damage or help regeneration of hepatic cell. 6 The leafy vegetables, *A. tricolor* comprises an excellent source of proximate and minerals, antioxidant leaf pigments, carotenoids, vitamins, phenolics and flavonoids 7. Natural antioxidants like leaf pigments, carotenoids, vitamins, phenolics and flavonoids have proven for health benefits as they detoxify ROS (ROS, reactive oxygen species) in the human body 7,8

In this study, the phytochemical profiles of the *amaranth tricolor*, and *A. tricolor* were investigated to evaluate the levels of phytochemical studies to optimal consumption in terms of alternative raw material for a novel food industry in the future.

MATERIAL AND METHOD:

➤ Collection of plant material^{9,10}:

The leaves of *Amaranthus tricolor* were collected in November 2023 from Beed district in India. The plant was authenticated by DR. Giri sir Department of Botany, from senior PVP COLLEGE PRAWARANAGAR dist. Ahmadnagar. The collected leaves were dried at room temperature.

➤ Chemical and Reagent^{11,12}: Chloroform, n-hexane, acetone, ethyl acetate were used as solvents for the process of extraction. The following chemicals were used for the phytochemical screening test: chloroform, sulphuric acid, Dragendoffs reagent, Molisch reagent, ammonia, acetic acid, hydrochloric acid, ferric chloride, copper acetate, million reagent, fehling's solution, sodium hydroxide, etc. All chemicals were of analytical grades.

METHODS^{13,14}:

- A. Total ash :** Take 2gram of drug in silica dish and incinerated at a temperature 400 to 450 degree celcius .when it is free carbon and formation of ash,cooled it and weight was taken.The percentage of drug was calculated as per formula.
- B. Acid -insoluble ash :**
Take the ash and boil it with the 25ml Hydrochloric acid for 5 min,filter it and collect the ash in crucible ,washed with hot water ,ignited,cooled,in a desiccator and weighted.The percentage of acid insoluble ash was calculated by air dried drug
- C. Water-insoluble ash:**
The ash was obtained as per the method described above for total ash.
Ash boiled for 5 minutes with 25 ml water.filtered and insoluble matter was collected in a grouch crucible ,washed with hot water and ignited for 15 minutes at a room temperature not exceeding 45 degree celcius.The weight of insoluble matter was deducted from weight of ash.
- D. Loss on drying:**
Weight powder about 1.5 gram into weighted porcelain dish.Dry it oven at 105 degree celcius until the weight is constant. Cool in a desiccator and observed as final weight. The loss in weight after drying is recorded as moisture.
- E. Water soluble extractive value:**
Take 4 gm of crude powder with 100 ml of chloroform water for 24hrs in a flask,shake for 6 hrs and stands for 18 hrs.then filter it.Take 25 ml of filtrate from it and evaporate it and dried it at 105 degree celcius and weighted.percentage of water-soluble extractive value was calculated by the air dried drug.
- F. Alcohol-soluble Extractive value:**
Take 4mg of powdered drug with 100ml of alcohol in a closed flask for 24 hr,and shake for 6hr and stands for 18 hrs.then filter it.Take 25ml of filtrate from,evaporate it and dried at temperature and weighted. And calculate the dried drug.

EXTRACTION OF PLANT MATERIAL^{15,16,17}:

The leaves of *Amaranthus tricolor*(L) was wash and cut.and they air-dried for one week under room temperature.Then dried plnt material was manually powdered finely and used for extraction.

In their four different extract (chloroform extract,ethyl acetate,acetone extract,n-hexane extract) in Soxhlet extraction method 25 gm of fresh immature leaf powder of *Amaranthus tricolor* placed in a thimble ,which is placed in a distillation flask containing solvent of 250 ml. after reaching an overflow level,the solution of thimble -holder is aspirated by a siphon tube which unloads the solution.

Extraction for CHLOROFORM

Extraction for N-HEXANE

Extraction for ETHYL ACETATE

Extraction for Acetone



RESULT AND DISCUSSION :

The powdered drug was subjected to extraction with various solvents such as petroleum ether, benzene, acetone, chloroform, ethanol and water by successive soxhlation and finally the marc was macerated with chloroform water I.P. Preliminary chemical analysis of various extracts of *Amaranthus tricolor* was done. From the result, it can be inferred that carbohydrates were present in ethanol and aqueous extracts; glycosides, phenolic compounds, flavonoids and saponins were present in chloroform, ethanol and aqueous extracts; and steroids were present in petroleum ether, benzene, acetone and chloroform extracts.

- Table 1 -Physicochemical Parametereters
- Table 2- Phytochemical Studies

SR.No	Physicochemical parameters	Results (% W/W)
1	Total ash	11.3
2	Acid-insoluble ash	5.9
3	Water-insoluble ash	4
4	Loss on drying	6.9
5	Water soluble extractive value	17
6	Alcohol soluble extractive value	6.3

Table 1

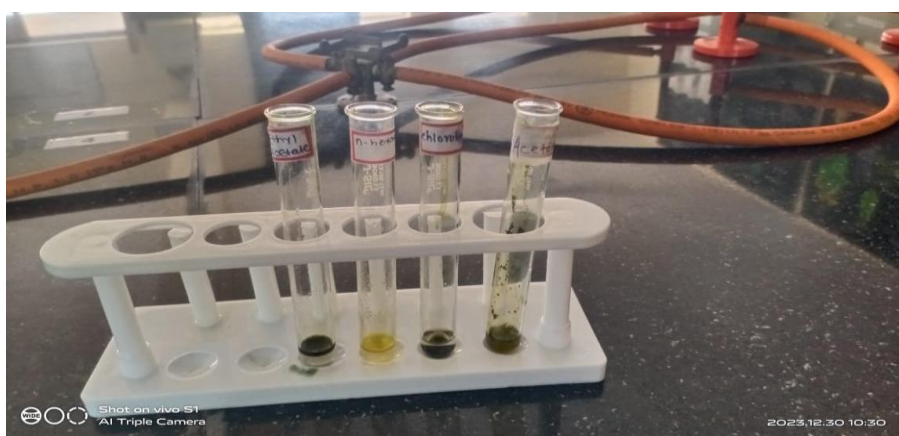
PRELIMINARY PHYTOCHEMICAL STUDIES :

SR. NO.	Test	Ethyl Acetate	CHLOROFORM	ACETONE	N-Hexane
1	For Alkaloids	+	+	+	+
2	Flavonoids	+	+	+	+
3	Saponins	-	+	-	+
4	Phenolic content	+	+	+	+
5	Steroids	+	-	-	-
6	Glycosides	-	+	+	+
7	Protein	-	+	+	+
8	Tannin	+	-	+	-
9	Terpenoids	+	-	+	+
10	Carbohydrates	-	+	+	+

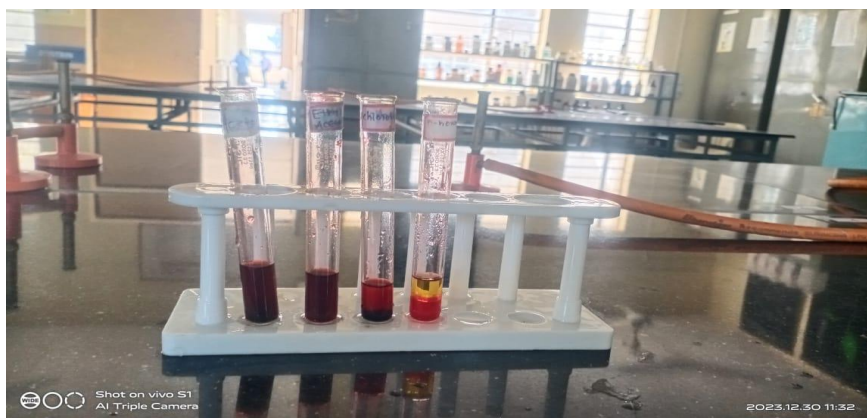
Table 2

1) Test for Alkaloids :

Dragendroffs Test : Few gm filtrate +1.2ml Dragendroffs reagent .Observation is a reddish brown color precipitate formed. All the solvents gives positive response to the determination of alkaloids



2) **Test for Flavonoid** : 1 gm extract was put into a test tube followed by addition of Hydrochloric acid and magnesium turnings. Development of an magenta red color is observed.All the four solvents are permiseabile to the test of flavonoids.



3) Test for Saponins: Few gm of extract mixed in a small amount of acetic unhydride with few drops of hydrochloric acid. It gives a yellow colour . Acetone and ethyl acetate solvent not showing positive response for the test of saponin.



4) Test for Phenolic content: Few gm of all the four solvents taken in separately test tubes adding a small amount of ferric chloride for observing a brownish red colour . all the solvents changes their colour after shaking the test tube. All four solvents show positive response for that test.



5) Test for Tannins : few gm of extract from all four solvents respectively in a separate test tube dissolved in a water adding few amount of sodium chloride and gelatin. observation is greenish black colour. Chloroform solvent not changes their colour hence, the test is not suitable for chloroform solvent.



6) Test for Glycosides : Few gm of extracts mixed with aquos sodium hydroxide .Pbseving yellow colour. Ethyl acetate not changes their colour due to absence of a glycoside content.



7) Test for Terpenoids : Few gm of extracts taken in a separately test tube adding chloroform in 0.5 ml adding conc.sulphuric acid for observation of red colour.Chloroform fails to turning their colour due to absence of the tepenoids content.



8) Test for carbohydrates: Molisch test performed for the test of carbohydrate.Take few gm of all four solvents separately in a test tube adding few drops of Molisch reagents in it.Obseving a ring formation in a test tube.Ethyl acetate fails to show the content of carbohydrates.



9) Test for Proteins: Few gm of solvents in a separately four test tubes adding few drops of an millions reagent for observing the reddish-brown colour.Ethyl acetate not changes their colour hence absence of protein content.



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REFERENCES:

1. J. B. Tucker, "Amaranth: The once and future crop," *BioScience*, vol. 36, no. 1, pp. 9–13, Jan. 1986
2. A. O. Olufolaji, F. O. Odeleye, and O. D. Ojo, "Effect of soil moisture stress on the emergence, establishment, and productivity of *Amaranthus cruentus* L.," *Agriculture and Biology Journal of North America*, vol. 1, pp. 1169–1181, Jan. 2010.
3. D. Prakash and M. Pal, "Nutritional and antinutritional composition of vegetable and grain amaranth leaves," *Journal of the Science of Food and Agriculture*, vol. 57, pp. 573–583, Aug. 1991
4. Cai, Y.; Sun, M.; Corke, H. Antioxidant Activity of Betalains from Plants of The Amaranthaceae. *J. Agric. Food Chem.* 2003, 51, 2288–2294. [CrossRef] [PubMed]
5. Sarker, U.; Oba, S. Leaf Pigmentation, Its Profiles and Radical Scavenging Activity in Selected *Amaranthus tricolor* Leafy Vegetables. *Sci. Rep.* 2020, 10, 18617. [CrossRef] [PubMed]
6. Vardharajan S. *The Wealth of India- A Dictionary of India Raw Materials and Industrial Products*. Revised edition Raw Materials, vol. 1A. New Delhi: NISCAIR, CSIR; 1985. p. 213-21.
7. Venskutonis PR, Kraujalis P. Nutritional components of amaranth seeds and vegetables: a sreview on composition, properties, and uses. *Comprehensive Review in Food Science and Food Safety*, 2013, 12:381–412.
8. Repo-Carrasco-Valencia R, Hellstrom JK, Pihlava JM, Mattila PH. Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (*Chenopodium quinoa*), kaniwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*). *Food Chemistry*, 2010, 120:128–133.
9. *Amarantus plus tricolor*, Available at www.pfaf.org/database/plants.php?. Received on 10th June, 2009.
10. *Plant Resources of South-East Asia No.8: Vegetables*. 1993, Wageningen, the Netherlands: Pudoc Scientific.
11. Harborne JB. *Phytochemical methods- A guide to modern techniques of plant analysis*. London / New York: Chapman and Hall, 1984.
12. AOAC. *Official Methods of Analysis*. 15th edn. Washington, DC: Association of Official Analytical Chemists, 1996.
13. Brain KR, Turner TD. Bristol: Wright-Scientetchnica; 1975b. *The Practical Evaluation of Phyto pharmaceuticals*; pp. 36–45. [Google Scholar]
14. *Medicinal and aromatic plants abstracts*, National institute of science communication and resources CSIR, New Delhi, vol-20-21, 1998-1999, p-537.
15. Agrawal MN, Anxiolytic activity of *Annona squamosa* leaf extracts in Mice, *Indian J. Pharma. Educ. Res.* 43(1), Jan-March-2009 , p-99.
- 16) Khandelwal K, *Practical Pharmacognosy, Techniques and experiments*, Nirali Publication, 2 nd edn, p-149-155
- 17) Samavardhana, K.; Supawitpattana, P.; Jittreotch, N.; Rojsuntornkitti, K.; Kongbangkerd, T. Effects of extracting conditions on phenolic compounds and antioxidant activity from different grape processing byproducts. *Int. Food Res. J.* 2015, 22, 1169–1179.