EVALUATION OF PHENOLIC COMPOUNDS FROM AMARANTHUS HYBRIDUS L. BY SPECTROPHOTOMETRIC METHOD.

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Abstract

The present study was undertaken to find out antioxidant content of leaves of Amaranthus hybridus. Collection of plant material were done from Melghat forest region, Dist.- Amravati, Maharashtra. Plants were identified with the help of the standard floras.1gm of leaves were used for determination of Phenolic compounds. Antioxidants have been reported to prevent oxidative damage caused by free radical and can be used in cardiovascular and anti-inflammatory diseases. Estimation of Bound Phenol, Total Phenol, Ortho-dihydric Phenol, Quinones, Tannins and Flavonol were done. Sufficient amount of phenolic compounds were observed in Amaranthus hybridus.

Keyword: Amaranthus hybridus, Phenolic compounds, Spectrophotometer.

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1.INTRODUCTION

Recently, many researchers have taken a great interest in medicinal plants for their phenolic concentrations and related total antioxidant potential (Katalinicet al.,2006).It is reported that some medicinal plants contain a wide variety of natural antioxidant, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity than dietary plants (Wong et al.,2006).

Polyphenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kahkonenet al., 1999). Herbs are used in many domains, including medicine, nutrition, flavouring, bever¬ages, dyeing, repellents, fragrances, cosmetics (Djeridaneet al.,2006). Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, antimutagenic effects and anticarcinogenic potential (Aaby, Hvattum, and Skrede, 2004). Crude extracts of herbs, spicesand other plant materials rich in phenolics are of increasing interest in the food industry as because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food.

A variety of plant secondary metabolites have been reported to act as antioxidants and amongst them phenolic compounds from a major group. There are several reports on the contribution of phenolic compounds to the antioxidant potential of different plant species (Caietal.,2004). Phenolics are able to scavenge reactive oxygen species (ROS) due to their electron donating properties. The antioxidant effectiveness in food depends on not only the number and location of hydroxyl group but also on factors such as physical location, interaction with other food components, and environmental conditions .In many studies, phenolic compounds demonstrated higher antioxidant activity than antioxidant vitamins and carotenoids (Re et al.,1999 and Veliogluet al.,1998). Amaranthus hybridus. A genus belonging to Amaranthaceae family, Erect,annual herbs; 1-1.5m tall; stem striate, branch in upper part, greenish purple, glabrous or hairy at the tip. Leaves ovate to, acute shortly decurrent at base, gradually narrowed upwards, obtuse, mucronate, Bracts and bracteoles ovate, 3-5mm long, long aristate. Tepals oblong 2.5mm long, green or

more often red purple, mucronate. Stamens longer than the tepals. Ovate oblong; styles recurved. Fruits urceolate, pale green in the lower half purple the upper half, circumsciss, seed globose, 1-1.2mm in diameter dark brown, shining.

Cultivated throughout the region for leaves used as vegetables and edible seeds.

Tribals, forest dwellers and rular rural folk living in interior area are still depend on their traditional knowledge to fullfill daily needs. About 3900 plant species are known to be used as subsidiary food /vegetables by tribals, however scanty information is available regarding their nutritional studies. Dry and fresh wild edible as well as medicinal herb used by Korkus of Melghat. (Bhogaonkar and Marathe,2008).

2. MATERIALS AND METHODS

2.1. Plant material

The extensive survey, identification and collection of plant from Melghat region was carried out. Plant identification was carried out with the help of floras (Dhore,1986;1998; Naik , 1998).

2.1.1. Preparation of plant material

Fresh leaves were collected and the dried in sunlight. After them powdered with mechanical grinder and stored in airtight container. Samples were powdered separately. 1gm of samples was taken for estimation of phenolic compounds gm/ µgm.

2.2. Methods

Estimation of Phenolics such as total phenol, Ortho-dihydric phenols, Bound phenol, Quinones, Flavonols and Tannins were done according to the methods prescribed by Thimmaiah (1999), which are given below.

2.2.1. Estimation of Bound Phenols

1gm sample was grind with the help of mortar and pestle with 5ml of SDS solution and centrifuge for 5 min and supernatant was discarded.

The residue was wash with once 5ml SDS solution, twice with 5ml of water, twice with 5ml of ethanol and twice with 5ml of diethyl ether (after each washing centrifuge and the supernatant was discarded). Allow the residue to dry and was suspended in 3ml of 0.5 M NaOH. It was kept overnight at room temperature. In the next morning it was Centrifuge and the supernatant was diluted 1:10 with 0.5 M NaOH (0.5 ml supernatant and 5 ml of 0.5 M NaOH).

Absorbance was measured at 290 nm against a reagent blank lacking only extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol. (Thimmaiah,S. R. 1999)

2.2.2. Estimation of Total Phenol

1gm of sample was grind with the help of mortar and pestle with 10mlof 80% ethanol. The homogenate was centrifuged for 20 minutes at 10,000 rpm. Supernatant was collected and evaporated to dryness. Then after dryness residue was taken and make up the volume with 5ml distilled water. 1 ml aliquot was Pipette out in test tube, and volume make up to3 ml with distilled water. To it 0.5 ml of Folin-Ciocalteu reagent was added. After 3 minutes, 2 ml of 20%Na2CO3solution was added into each tube.

Mixed thoroughly and tubes was kept in boiling water for 1 minute, then allowed to Cool and absorbance at 650 nm was measured against reagent blank.

Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol. (Thimmaiah S. R. 1999)

2.2.3. Estimation of Ortho- dihydric phenols.

1gm sample was grind with the help of mortar pestle with 10ml of 80% ethanol Centrifuge it for 20 minutes at 10,000 rpm and supernatant was collected. Then supernatant was evaporated to dryness. Then after dryness residue was taken and make up the volume with 5ml distilled water. 1 ml of aliquot was pipette out in a test tube, to it 1ml of 0.05 NHCL, 1 ml of Arnow's reagent, and 10ml of distilled water and 2ml of 1N NaOH was added. Absorbance was measured at 515 nm against a reagent blank lacking only extract.

Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol. (Thimmaiah S. R. 1999)

2.2.4. Estimation of Quinones

1gm sample was grind with the help of mortar and pestle with using chilled phosphate buffer (5ml for each gm of tissue). The supernatant was collected and Centrifuged for 30 minutes this was used as enzyme extract. 3ml of buffer, 3mlofstandardcatechol and 1.5 ml of enzyme extract was pipette in a test tube. It was shaken gently and incubated in water bath. 4ml of TCA (Trichloro acetic acid) reagent (without ascorbic acid) to one and 4ml of TCA reagent (with ascorbic acid) was added. Precipitate was filtered. Absorbance was measured at 400 nm against a reagent bank lacking only extract.

Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of working standard catechol. (Thimmaiah S. R. 1999)

2.2.5. Estimation of Tannins

Vanillin hydrochloride method was used.

1 gm of sample was mixed in 10ml methanol after 20-28 hrs. Centrifuged and supernatant was collected. Pipette out 1ml supernatant into test tube and quickly 5ml of vanillin hydrochloride reagent was added and mixed. After 20 min absorbance was read at 500nm. A reagent blank was prepared with vanillin hydrochloride reagent alone. A catechin standard graph was prepared from working standard (100µg/ml) of catechin and amount of tannins was calculated. (Thimmaiah,S. R. 1999).

2.2.6. Estimation of Flavonols

1gm sample was grind with the help of mortar and pestle with 10ml of ethanol and the supernatant was collected by centrifugation for 20 minutes. The supernatant was evaporated to dryness; then the residue was dissolved in 5 ml distilled water. 1ml of extract was pipette out into 25ml conical flask and 1 ml of distilled water was added.

Then 4ml of vanillin reagent was added from a burette rapidly within 10-15 sec to flask. A and4ml of 70% H2SO4 to flask B.A blank was prepared in flask C containing 4 ml of vanillin reagent and 2ml of distill water. Both flasks A and B was shaken in a water bath at the temperature below 350C.Keeping the flasks at room temperature for exactly 15min.Absorbance was measured flask A, B and C at 500 nm against 47%. H2SO4(flask D).

The absorbance of the flasks B and C was subtracted from that of A. The flavonol content was calculated using a standard curve prepared from phlorogucinol or kaempferol(100 µg/ml). (Thimmaiah, S. R. 1999)

A standard graph was obtained by plotting concentration on X- axis and the corresponding values of absorbance along Y- axis on a graph paper resulting a straight line which passes through the origin and maximum points of standard reading. It is used to quantify the amount of a given compound present in an unknown sample whose absorbance value is matched against that of standard along Y-axis and a corresponding concentration could be read off along X-axis.. (Thimmaiah, S. R. 1999)

3. RESULT AND DISCUSSION

1. TABLE Amaranthus hybridus.(leaves) showing the biochemical

Plant material and part used for estimation	Bound phenol		Total Phenol		Orthodihydric Phenol		Quinone		Tannin	
	Absorbance at(290nm)	µgm/gm	Absorbance at(650 nm)	µgm/gm	Absorbance (515nm)	µgm/gm	Absorbance (400nm)	µgm/gm	Absorbance (500nm)	µgm/gm

Amaranthus hybridus.(leaves)	0.10	16400	0.35	2300	0.01	350	0.05	80	0.25	550

2.TABLE Amaranthus hybridus.(leaves) showing Flavonol

Part used for estimation	Flavonol Absorbance at (500nm)			A- (B+ C)	Coincidence with concentration on standard graph
	Flask A	Flask B	Flask C		
Amaranthus hybridus L.(leaves)	1.39	0.023	0.097	1.27	1.08

Highest amount of Bound phenol (16,400µgm/gm) and Total phenol (2300µgm/gm)was observed in Leaves of Amaranthus hybridus and while lowest content of Flavonol (1.08µgm/gm). However there is significant amount of quinone was found (80µgm/gm) and Tannin (550µgm/gm).

350 µgm/gm Orthodihydric phenol was observed in leaves of Amaranthus hybridus.

Phenolic compounds and flavonoids have been reported to be associated with anti-oxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals. (Rice-Evans et al.,1997). The nitric oxide scavenging activity of flavonoids and phenolic compounds are known (Kim H. et al., 2002). Phenols are present in food, they may have an impact on health and most are known to have an antioxidant activity. (Demitrios 2006) .

Phenols and Polyphenolic compounds such as flavonoids are widely found in plant sources and they have been shown to posse's significant antioxidant activities (Van Acker S. et al.,1996).

Medicinal plants contain some organic compounds which provide definite physiolgical action on the human body and these bioactive substances include tannins, alkaloids, flavonoids, carbohydrates,herbenoids and steroids.(Edogaet al., 2005; Mann 1978).

4.CONCLUSION

This study reveals that the leaves of Amaranthus hybridus L. contain rich Bound phenol, Total phenol

Quinone and Tannins which are known to posses good source of antioxidant activity and anti-inflammatory activity. Amaranthus hybridus L. was used as vegetable in melghat. The plant is a good source of antioxidant.

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