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A COMPARTIVE STUDY ON ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF AMARANTHUS ROXBURGHIANUS AND AMARANTHUS TRICOLOR

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ABSTRACT

Amaranthus roxburghianus and Amaranthus tricolor are traditionally well-known plants with outstanding therapeutic properties, and is used mostly in treating different diseases in India. Amaranthus roxburghianus is wild plant whose leaves and tender shoots and can be edible and are commonly used as a leafy vegetable. And used as iron tonic,its extracts or herbal formulations are rich in alkaloids ,used in traditional chinese and ayurvedic medicine. And its root combination with piperine is used in the effective treatment of inflammatory bowel disease and commonly, called as Prince's feather (English) schirikoora (Telugu). It is also distributed in Occasional weed of waste lands and cultivated lands.Amaranthus tricolor commonly called as red amaranthus or Joseph's coat (English) and thotakura(telugu) and this plant is often cultivated in tropical and warm temperature regions,especially in Asia, for its edible leaves and seeds. The present study has to perform the antioxidant activity of both plants and compare the both plants of antioxidant activity.

Keywords: comparative Amaranthus Extract Tricolor Roxburghianus Amaranthus Antioxidant

INTRODUCTION

Antioxidants are the compounds are part of our lives. Antioxidants are the molecule that inhibits the oxidation of other molecules. Oxidation is the process of chemical reaction that transfers electrons or hydrogen from substances to an oxidising agent. Antioxidants neutralizes or destroys "reactive oxygen species" or free radicals before they damage cells, caused by the oxidation process .An imbalance between oxidants and antioxidants potentially leading to damage is termed as "Oxidative stress. Changing in the environmental conditions are responsible of free radicals, which plants have to deal with them in order to survive. Reactive oxygen species, such as singlet oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide, are highly reactive, toxic molecules, which are generated normally in cells during metabolism [1]. They cause severe oxidative damage to proteins, lipids, enzymes and DNA by covalent binding and lipid peroxidation, with tissue injury. Natural antioxidant agents have the ability to scavenge free radicals. Free radical shave been implicated in the development of a number of disorders, including cancer, neurodegeneration and inflammation, giving rise to studies of antioxidants for the prevention and treatment of diseases. The presence of antioxidants such as phenolic, flavanoids, tannins and proanthocyanidins in plants may provide protection against a number of diseases; for example, ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders [2]. Medicinal plants are being investigated to evaluate antioxidant properties, and the demand for natural antioxidants and food preservatives is increasing Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by the emergency of multidrug-resistant pathogens. Many infectious diseases have been with herbal remedies throughout the history of mankind. Natural medicinal plants have the capable to develop better drugs against microbial infections.India is one of the richest countries in the world in regarding to genetic resources of medicinal plants.

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Plants have been widely considered as the traditional medicines throught the world for thousands of years and continue to provide new remedies to human kind. In recent years, secondary plant metabolites (phytochemicals) previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [3].

RATIONALE OF THE STUDY

Antioxidant is a chemical that prevents the oxidation of other substances. Naturally occurring antioxidants differ in their composition, their physical and chemical properties, their mechanism and site of action is differing. Plants have long been a source of exogenous (i.e., dietary) antioxidants. It is believed that two thirds of the world's plant species have medicinal importance, and almost all of these have excellent antioxidant potential [4]. The interest in the exogenous plant antioxidants was first evoked by the discovery and subsequent isolation of ascorbic acid from plants. since then, the antioxidant potential of plants has received a great deal of attention because increased oxidative stress have been identified as major causative factor in the development and progression of several life threatening diseases, including neurodegenerative and cardiovascular disease. In addition supplementation with exogenous antioxidants or boosting of exogenous antioxidant defenses of endogenous antioxidant defenses of the body has been found to be a promising method of countering the undesirable effects of oxidative stress [5].

PLANT PROILE

Amaranthus roxburghianus:

Amaranthus roxburghianus is one of the traditionally well-known plants with outstanding therapeutic properties, and is used mostly in treating different diseases in India. Amaranthus roxburghianus is commonly, called as Prince's feather (English) schirikoora (Telugu). It is also distributed in Occasionalweed of waste lands and cultivated lands (Kung et al,. 1935) [6].

Plant description

Annual herbs, stem are 20-50 cm tall, glabrous. Leaves are alternate; petiolate; lamina ovate- rhombic, 2-5 × 2.5cm, base cuneate, glabrous. Flowers are few. Sparsely clustered at the axils, and are in terminus. Bracts and bracteoles subulate, 2mm, abaxially with a distinct midvein, apex long pointed. Tepals -5,lanceolate, long pointed. Stamens are shorter than perianth. Stigmas are 3. Utricles ovoid, Sub equal to perianth, circumscissile, seeds brownish block, sub globose [7]

Use: Leaves are used as leafy vegetables

Amaranthus Tricolor

Amaranthus tricolor L(Amaranthacea) is an ornamental plant and in Indian tradition medicine, the plant is used for the treatment of variety of ailments like cough, throat, infections, toothache, ecezema, piles, diarrhoea, gonorrhoea, and impotence(Aneja et al 2011).[8].

PLANT DESCRIPTION:

Amaranthus tricolor is usually much branche,vigorous,erect or ascending annual plant with a stem,growing up to 125 cm tall.a major leaf crop in tropical Asia,where it is widely consumed and often solid in local market

Use: Leaves are uesd as leafy vegetables

Externally used to treat inflammation and internally diuretic.[8]

MATERIALS AND METHODS

Collection and preparation of samples

Amaranthus roxburghianus and *Amaranthus tricolor* plant was obtained from nearby areas of Piler and Chittoor. The collected plants were identified and authenticated by plant Taxonomist Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati with voucher number 0788 and 0793.Fresh aerial parts of the plant was collected, cleaned and washed under running water to remove soil and dirt, then air dried. The dried samples were milled with an electric blender before being ground into powder and stored in desiccators until required for analysis [9]

Phytochemical screening

Preliminary Phytochemical of *Amaranthus roxburghianus* was screened with different solvent extracts and the study was performed for the presence of alkaloids, Saponins, flavanoids, carbohydrate, glycosides, quinones, tannins, proteins, sterols and phenols according to the standard methods described by Harborne. The tests were assessed depending upon the visual observation of colour change of a specific precipitate after the addition of specific reagents [10]

Molisch's test:

Molisch test is a general test for identification of all carbohydrates,disaccharides, polysaccharides. In this test take 2ml of sample in dry test tube. Take 2ml of distilled water in another tube as control. Add 2-3 drops of Molisch's reagent to the solution. Gently pipette 1ml concentrated sulphuric acid along the side of the tube so that two distinct layers are formed. Observe colour change at the junction of two layers. Appearance of purple colour indicates the presence of carbohydrates.

Liebermann burchard's test

The test is used to determine Phytosterols. The chloroform extracted solution was treated with few drops

of acetic anhydride. Boil and cool. Add concentrated sulphuric acid formation of bluish green colour is produced.

Ferric chloride test:

The test is used to determine tannins. Treat the extract with few drops of ferric chloride solution. Formation of transient greenish to black color indicates the presence of tannins.

Dragendroff's test

The test is used to determine alkaloids. To 1 ml of test solution 2 ml of Dragendroff's reagent was added and mixed. To this 2 ml of dilute HCL was added. Orange precipitate is obtained.

Foam test:

3ml of test solution was taken in a test tube and vigorously shaken. Formation of foam indicates the presence of Saponins.

Liebermann's test:

The test is used to determine steroids. 2mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour is obtained.

Sodium hydroxide test:

The test is used to determine Flavanoids. The test solution was treated with sodium hydroxide solution. A canary Yellow colour is obtained

Millions reagent:

The test is used to determine protein. In this test take 2ml of extract add million's reagent a white precipitation is formed, which turns brick red on heating.

ANTIOXIDANT ACTIVITY

The human body has a complex system of natural enzymatic and non enzymatic antioxidant defences which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for the causing of oxidation reaction. Various methods are used to investigate the antioxidant reaction to evaluate the antioxidant property [9]

Antioxidant activity has two methods. They are:

In vitro method.
In vivo method.

METHODS IN INVITRO MODEL

Antioxidant activity should not be concluded based on a single antioxidant test model and in practice several in vitro test procedures are carried out forevaluating antioxidant activities with the sample of interest. Generally in vitro antioxidant tests using free radical traps are relatively straight forward to perform.

They are 12 types:[10]

- DPPH scavenging activity
- Hydrogen peroxide scavenging (H202) assay
- Nitric oxide scavenging activity:
- Peroxynitrite radical scavenging activity
- Trolox equivalent antioxidant capacity (TEAC)method / ABTS radicalcation decolourization assay
- Total radical –trapping antioxidant parameter (TRAP) method
- ferric reducing- antioxidant power (FRAP) assay
- Super radical scavenging activity (SOD)
- Hydroxyl radical scavenging activity
- Hydroxyl radical averting capacity (HORAC) method
- Oxygen radical absorbance capacity (ORAC) method
- Reducing power method (RP)
- Phosphomolybdenum method
- Ferric thiocyanate (FTC) method
- Thiobarbituric acid (TBA) method
- N,N -dimethyl-p-phenylenediaminedihydrochlorid (DMPD)method
- □-carotene li-leic acid method /conjugated diene assay
- Xanthine oxidase method
- Cupric ion reducing antioxidant capacity (CUPRAC) method
- Metal chelating activity.

In vivo models:

For all in vivo methods the samples that are to be tested are usually administered to the testing animals (mice, rats, etc.) at a definite dosage regimen as described by the respective method. After a specified period of time, the animals are usually sacrifice and blood or tissues are used for the assay [11]

There 9 types

- Ferric reducing ability of plasma
- Reduced glutathione (GSH) estimation
- Glutathione peroxidase (GSHPX) estimation
- Glutathione-s-transferase (GST)
- Superoxide dismutase (sod) method
- Catalase (CAT)
- □-Glutamyl transpeptidase activity (GGT) assay
- Glutathione reductase (GR) assay
- Lipid peroxidation (LPO) assay.[12]

FRAP

Ferric reducing antioxidant potential[FRAP] of the given extract was measured according to method proposed by Benzie and Strain.

FRAP reagent proposed by mixing in 25ml acetate buffer[30mM:PH3.6],2.5ml TPTZ solution [10mm] and 2.5ml ferric chloride solution[20mM]

The mixture was incubated for 15 min at 37 before use.

Ascorbic acid[vitamin c] was employed a standard in this essay and its calibration curve obtainedby using its concentration ranging from6.25ug/ml to 100ug\ml in methanol.

To 2.85 ml FRAP reagent in a test tuube,150ul given test extract[1mg/mlin menthol] or standardwas added.

The mixture was incubated for 30 min in dark and its absorbance was measured at593nm.The blank contained as equal volume of methanol instead of test compounds.

The results were reported to as ug of acorbic acid equivalent. [AAE] per ml.[13]

ABTS+Decolorization activity

1. Antioxidant activity of amaranthus extracts per ABTS+Decolorization assay was measured usingthe method reported by Re.et.al.
2. The working solution of ABTS+radical was prepared by adding ABTS[9.5ml,7mM] to potassium persulfate[245ul,100mM] and rasing the volume to 100ml with distilled water.
3. The solution was kept in the dark at room temperature for 18 th and then diluted with potassium phosphate buffer [0.1M,PH7.4] to an absorbance of 0.70[at 734nm. given test samples were prepared in methanol with different dilution.
4. A sample 10ul was placed in a test tube and mixed thorough with 2.99ml ABTS radical working solution absorbance of the resulting clear mixture was recorded at734nm.
5. The percent antioxidant activity of the sample was determined by using the following formula.%Antioxidant activity =100-[As-Ab]\[AC-AB]x100.

Where Ab,Ac andAs are the absorbance of blank,control samples was prepared contaning.

1. BHT was used as standard and menthanol as a blank ,control and sample was prepared containing the same volume without any extract.
2. The IC50 values was determined by using linear regression equation y=mx+c.here y=50,M and c values were derived from the regression graph trendline.[14]

DPPH

Freshly prepared DPPH solution in methanol was taken in test tubes and extracts were added followed by serial dilutions (6.25µg/ml to 100µg/ml) to every test tube to achieve the final volume of 1ml.
Incubate the Test Tubes for 30 min at RT

Quercetin was used as standard and Methanol as a Blank. Control sample was prepared containing the same volume without any extract (DPPH alone).Read the absorbance on a spectrophotometer at 517nm.[15]
The % radical scavenging activity of the given compounds was calculated using the following formula:

DPPH Radical Scavenging Activity= [(Abs of Control-Abs of Sample)/Abs of Control] × 100

The IC_{50} value was determined by using linear regression equation i.e. Y =Mx+C. Here, Y = 50, M and C values were derived from the linear.

Antioxidant activity

ABTS+ Decolorization activity of the Test compounds:

The Observations in Statistical data of ABTS•+ Decolorization potential Study by Spectrophotometer/ELISA Reader suggesting us that given test compounds viz Quercetin, A.roxburghianus and A.tricolor showed IC50 concentrations at 6.85ug/ml, 19.06ug/ml and 22.95ug/ml respectively.[16]
Among given test compounds, both A.roxburghianus and A.tricolor exhibited satisfactory ABTS•+ Decolorization potential property with the considerable low IC50 values compared to the Standard Drug, Quercetin which exhibited 6.85ug/ml used in the study.

The obtained results of ABTS•+ Decolorization potential study given test compounds indicating that the A.roxburghianus and A.tricolor extracts havingsignificant ABTS•+ Decolorization potency on dose dependent manner.

A.roxburghianus showed significant ABTS•+ Decolorization potential propertiesthan A.tricolor due to its low IC50 value [17].

Iron ion chelating activity of the Test compounds

The Observations in statistical data of Iron ion study by Spectrophotometer/ELISA Reader suggesting us that given test compounds viz Quercetin, A.roxburghianus and A.tricolor showed IC50 concentrations at 50ug/ml,61.75ug/ml and 65.84ug/ml respectively. Among given test compounds, both A.roxburghianus and A.tricolor exhibited satisfactory Metal ion chelating potential property with the considerable low IC50 values compared to the Standard Drug, Quercetin which exhibited 50ug/mlused in the study.[19].

DPPH Radical Scavenging Study of the Test compounds

The Observations in Statistical data of DPPH RSA Study by Spectrophotometer/ELISA Reader suggesting us that given test compounds viz Quercetin, A.roxburghianus and A.tricolor showed IC50 concentrations at 14.56ug/ml, 32.19ug/ml and 40.96ug/ml respectively.

Among given test compounds, both A.roxburghianus and A.tricolor exhibited satisfactory DPPH Radical Scavenging potential property with the considerable low IC50 values compared to the Standard Drug, Quercetin which exhibited 14.56ug/ml used in the study.[20].

Ferric Reducing Antioxidant Potential (FRAP)of test compounds:

The Observations in Statistical data of FRAP Study by ELISA reader suggesting us that given Test Compounds viz., Quercetin, A.roxburghianus and A.tricolor showing effective Ferric reducing antioxidant potential (FRAP) potency compared to the Standard drug, Ascorbic acid used for the study.

Figure 1. Amaranthus roxburghianus

Figure 2. Amaranthus Tricolor

Table 2. Classification of Plant

SCIENTIFIC CLASSIFICATION	
class	Magnolipsoda
Division	Magnoliophyta
family	Amaranthacea
Kingdom	Plantae
Order	Carryophyllates
Genus	Amaranthus.L
Species	Amaranthus tricolor
BINOMINAL NAME	
Amaranthus tricolor	

Table 3. Preliminary phytochemical screening of Amaranthus roxburghianus and Amaranthus tricolor

S.No		TESTS	A.roxburghianus	A.tricolor
1		**Test Of Alkaloids**		
	1.1	Dragondroffs	+	+
	1.2	Hagers	-	-
	1.3	Mayers	-	-
	1.4	Wagers	+	-
2		**Test For Glycosides**		
	2.1	Legals test	-	-
	2.2	Raymonds	-	-
	2.3	Baljet test	-	-
	2.4	Bromine water	-	+

3		TEST FOR SAPONINS		
	3.1	Foam test	+	+
4		TEST FOR CARBOHYDRATES		
	4.1	Molish test	+	-
	4.2	Fehlings test	+	+
	4.3	Benedicts test	-	+
5		TEST FOR TANNINS		
	5.1	Gelatin	-	-
	5.2	Fecl3	+	+
6		TEST FOR FLAVANOIDS		
	6.1	Shinodas	+	+
	6.2	Zinc dust test	+	+
	6.3	Alkaline reagent	+	+
7		TEST FOR STEROIDS AND TERITERPENOIDS		
	7.1	Liberman buchard	+	+
	7.2	Salkowski	-	-
8		TEST FOR PROTEINS		
	8.1	Biuret	-	+
	8.2	Millons	+	-
	8.3	Ninhydrin	-	-
9		TEST FOR FATS AND FIXED OILS		
	9.1	Spot test	-	-
	9.2	Saponification test	-	-

('+') indicates presence; while ('–') stands for absence

Table 3. ABTS+ Decolorization activity of the Test compounds

Sample code	IC50(ug/ml)
Quercetin	6.85
A.roxburghianus	19.06
A.tricolor	22.95

Table 4. Iron ion chelating activity of the Test compounds

SAMPLE CODE	**IC50(ug/ml)**
Quercitin	50
A.roxburghianus	61.75
A.tricolor	65.84

Table 5. DPPH Radical Scavenging Study of the Test compounds

SAMPLE CODE	**IC50(ug/ml)**
Quercetin	14.56
A.roxburghianus	32.19
A.tricolor	40.96

Table 6. Ferric Reducing anti oxidant potential (FRAP) of test compound:

Sample code	6.25	12.5	25	50	100
Quarcetin	16.6667	66.8333	83.1667	113.5	142.667
P.Roxburghianus	3.16667	9.83333	16.7	39.6667	76.6667
P.Tricolor	9.16667	23	43	53.1667	93.6667

DISCUSSION:

Amarathus roxiburghianus and Amaranthus tricolor contain alkaloids,glycosides , saponnins, carbohydrates ,tannins, flavanoids ,steroids and triterpinoids and proteins.

The obtained results of ABTS•+ Decolorization potential study given test compounds indicating that the

A.roxburghianus and A.tricolor extracts having significant ABTS•+ Decolorization potency on dose dependent manner. A.roxburghianus showed significant ABTS•+ Decolorization potential properties than A.tricolor due to its low IC50 value.

The absorbance of ferrous and ferrozine complex was decreased dose- dependently, which means that the chelating activity was increased on increasing concentration from 6.25-100µg/mL. The highest chelating activity of A. roxburghianus and A. Tricolor is at 100µg/mL with 63.14% and 74.71%, respectively. However, the chelating activity of A. Tricolor extract still can be improved by increasing the concentration. The smaller the IC50 value, the higher the metal chelating activity of the compound / extract. This reveals that the A. roxburghianus and A. Tricolor extracts were moderate metal chelating agents as compared to Quercetin. The ferrous ion- chelating activity was shown by A. roxburghianus and A. Tricolor and positive control, Quercetin with IC50 values of 61.75µg/mL, 65.84µg/mL and 50µg/mL respectively.

The obtained DPPH radical scavenging results of given test compounds indicating that the A.roxburghianus and

A.tricolor extracts having significant DPPH radical scavenging potency on dose dependent manner. A.roxburghianus showed significant DPPH radiacal scavenging potency than A.tricolor due to its low IC50 value.

Comparative FRAP activity of given test compounds with different concentrations ranging from 6.25ug/ml to 100ug/ml and all the compounds showing significant FRAP potential in dose dependent manner

CONCLUSION

Plant serve as the source of rich phytochemical diversity that possesses important biological and pharmcological activities. Amaranthus is an important tradiional widely used in folk medicine for centuries.This study suggest that Amaranthus roxburghainus and Amaranthus tricolor exbihit great potential for antioxidant activity and may be useful for their nutritional and medicine function. when compared to Amaranthus tricolor Amaranthus roxburghianus will have more antioxidant properties.

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Nil

CONFLICT OF INTEREST

No interest

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