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**A Study of Phytochemical Screening of Total Phenolic and Flavonoid  
Content In *Amaranthus hypochondriacus* L. Grains**

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**ABSTRACT**

Methanol extracts of the dried seeds of *Amaranthus viridis* were collected and used for phytochemicals and antioxidant activity. The extract yields from seeds ranged 5.5 - 6.1 and 2.42% - 3.72% w/w, respectively. Phytochemical investigation of this plant determines that tannins (6.07% - 5.96%), alkaloids (13.14% - 11.42%). The ex-tracts also contained appreciable levels of total phenolic contents (2.81 - 3.61 GAE, g/100 g), total flavanoid contents (18.4 - 5.42 QE, g/100 g) and DPPH free radical scavenging activity. The results of this study suggest the possibility of using the methanolic extracts in treating the diseases caused by the test organisms.

**INTRODUCTION**

In developing countries such as Burkina Faso, a large part of the population has the habit of consuming traditional food plants. Recent phytochemical analyses have shown that most plants consumed contain several health protecting nutrients (nutraceuticals). The popularity of nutraceuticals and traditional medicines are soaring as consumers take a keener interest in health and nutrition. As markets for these products develop, tighter regulations are being introduced to ensure quality, efficacy and safety. Ethnobotanical and traditional food plant investigations in the central region of Burkina Faso have shown that approximately thirty Caryophyllales species are widely and frequently used in traditional medicine to treat various kinds of diseases (e.g., malaria, fever, pain, hepatic disorders, nervous system issues, cancers, and cardiovascular diseases) and are also consumed as food plants. Phytochemical screening (qualitative and quantitative) by laboratory standard means should allow for the validation of their nutraceutical potential. The present study concerns *Amaranthus cruentus* L. and *Amaranthus hybridus* L., two species from the Amaranthaceae family (Caryophyllales). *Amaranthus* spp. were of great importance in pre-Colombian American people's in particular, *A. cruentus* and *A. hybridus* have a high

nutritional value. The consumption of *A. cruentus* products is advised for patients with celiac disease and, therefore, also for diabetic persons. *hybridus* has been used traditionally for the treatment of liver infections and knee pain and for its laxative, diuretic, and cicatrisation properties the products are used particularly for stomach aches, diarrhoea, and dysentery. *A. hybridus* leaves are used as a vegetable, and sauces prepared from this plant are recommended for convalescent patients. These two species are reputed to promote health and a long shelf life.

### **RAJGIRAA (Amaranth)**



- **Synonym-** Amaranthus
- **Biological source-** Amaranthus Viridis Linn.
- **Family-** Amaranthaceae
- Rajgira Seeds are a type of crop that is also known as Amaranthus are used in the manufacturing of Chikkies, Lai, Patti and Laddo. The Rajgira Seeds are a rich source of protein and nutrients. Jagdish Rice Mill is one of the renowned Rajgira Seeds suppliers, which are widely praised by clients for their high nutritional value.

#### ➤ **Chemical constituents-**

**Energy-** 1554Kj(371kcal)

**Carbohydrates-**65.25g

Starch-57.27g

Sugars-1.69g

Dietary fiber-6.7g

#### **Fat-**

Saturated-1.459g

Monounsaturated-1.685g

Polyunsaturated-2.778g

#### **Protein-**

Tryptophan-0.181g

Lysine-0.747g

Histidine-0.389g

**Vitamins-**

Thiamine(B1)-0.116g

Vitamin C-4.2g

Vitamin E-1.19g

**Minerals-**

Calcium-159mg

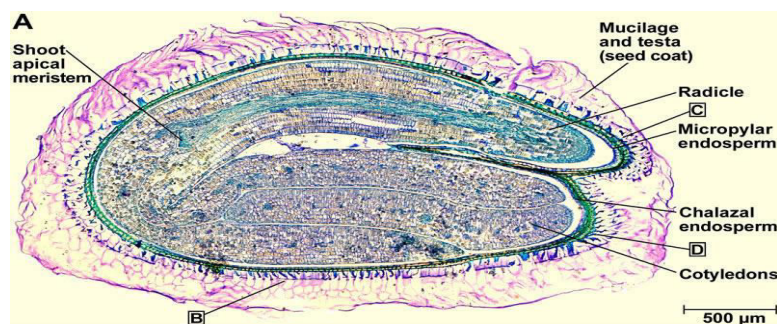
Iron-7.61mg

Zinc-2.89mg

**Other constituents-** Water-11.13g

**Macroscopy**

The following macroscopic characters for the fresh parts of plant were noted: Size and shape colour, surfaces, venation, the apex, margin, base, lamina, texture, odour and taste (Wallis, 1985; Evans, 2002). Microscopy The anatomies of the root, stem and leaf were determined by a standard method (Wallis, 1985; Evans, 2002). The outer epidermal membranous layer of leaf (in fragments) were cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence/absence of epidermal cells and stomata (type and distribution) were observed. The transverse sections of the fresh leaf, stem and root as well as a small quantity of the powdered plant were also cleared, mounted and observed under a compound microscope (Clark, 1960; Bokhari, 1971; Cotton, 1974; African Pharmacopoeia, 1986; Subrahmanyam, 1996). Chemomicroscopic examination Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques (Evans, 2002). Phytochemical investigation Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins, cardiac glycosides, alkaloids, saponins, anthracene derivatives and cyanogenetic glycosides (Johansen, 1940; Brain and Turner, 1975; Ciulei, 1981; Harborne, 1992; Evans, 2002). Quantitative investigation Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein – islet number and veinlet termination number were carried out on epidermal peelings (British Pharmacopoeia, 1980)



## Materials and Methods

### Collection and Pretreatment of Plant Material

Fully matured leaves and seeds of *Amaranthus viridis* L. were collected from the fields of Lalliyar, which is a city in the Islampur Dist- Sangli in India. These plants were identified by the Department of Krishna Laboratory, Karad. Fully matured leaves and seeds from the plant were selected because there is maximum metabolism in fully matured leaves and seeds as compared to young leaves and seeds. Specimens were dried at room temperature and stored in polyethylene bags at 4°C.

### Phytochemical Analysis

Phytochemical screening for the presence of Tannins, alkaloids, glycosides, flavonoids, and phenolic was performed using standard procedures

### Qualitative Analysis of Phytochemicals

#### 1) Alkaloids

The extracts were evaporated to dryness and the residues were heated on a boiling water bath with 2% Hydrochloric, cooled, filtered and treated with the Mayer's reagent. The sample was then observed for the presence of yellow precipitation or turbidity.

#### 2) Flavonoids

1.5 ml of 50% methanol was added to 4 ml of extracts. After warming add magnesium filings followed by few drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoid.

#### 3) Tannins

A portion of the extract was diluted with distilled water in a ratio of 1:4 and few drops of 10% ferric chloride solution were added. A blue or green color indicates the presence of tannins.

### Chemical and Reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteu reagent, sodium nitrite, Linoleic acid, butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Company (St. Louis, USA) and anhydrous sodium carbonate, methanol, ethanol, diethyl ether, n-butanol, sulphuric acid, fehling solution, amyl alcohol, chloroform, olive oil, magnesium turnings, ammonia, ferric chloride, sodium nitrite and ammonium hydroxide used were obtained from dept. of pharmaceutical chemistry lab.

Instrument use- uv spectroscopy (Jasco V-630)

### Extraction Process:-

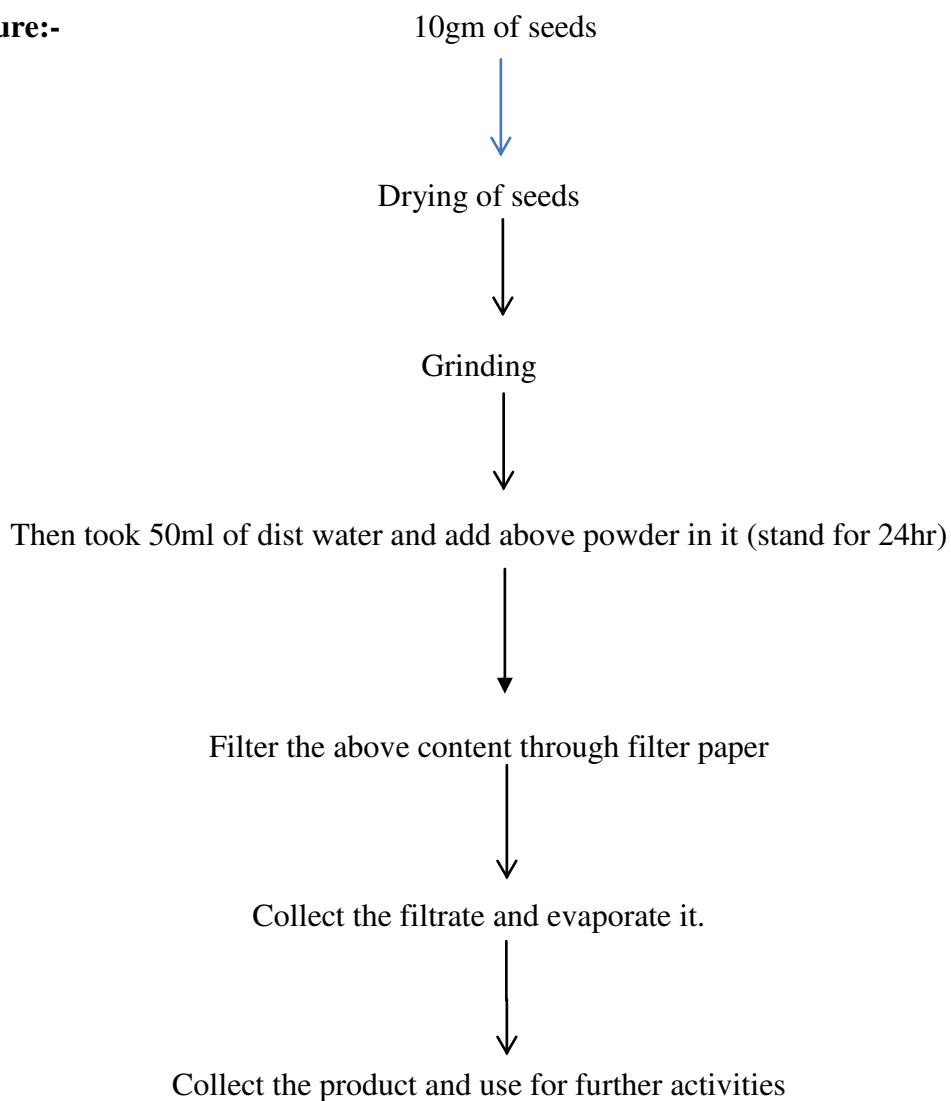
- 1) Aqueous extract- it is carried out by maceration method..
- 2) Methanolic extract-it is carried out by maceration method

#### 1) Aqueous extract / Phenolic extract

##### Maceration:-

The maceration in water shouldn't be prolonged for too long as this can present the fungal contamination which does not occur in alcohol or hydro alcoholic solution. Total maceration time depends on type of plant or part thereof active ingredient to extract. The most commonly used ratio is 1:20 herb/liquid.

##### Procedure:-



### **Determination of total phenolic content -**

The concentration of phenolics in plant extracts was determined using spectrophotometric method. [9]Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). One millilitre of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) 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  $\mu\text{g}/\text{ml}$ ) 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 extract.

### **Determination of tannin Content –**

The tannins were determined by 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 %  $\text{Na}_2\text{CO}_3$  solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100  $\mu\text{g}/\text{ml}$ ) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract.

### **Determination of Total flavonoid content -**

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml 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  $\mu\text{g}/\text{ml}$ ) 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 an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

## **Antioxidant Activity**

### **1. Total Phenolic Contents (TPC) & Total Flavonoid Contents (TFC)**

Total phenolic contents (TPC) were determined using the Folin-Ciocalteu reagent method and Gallic acid was used as Gallic acid Equivalent (GAE). Total flavonoid contents (TFC) in the leaf and seed extracts were determined following the modified procedure and Quercetin was used as standard as Quercetin Equivalent (QE).

## 2. DPPH Radical Scavenging Assay :

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay was carried out spectrophotometrically [24]. The percent inhibition was calculated as:

$$\% \text{Inhibition} = (1 - A/A_0) \times 100$$

Where A blank the absorbance of the control reaction (containing all reagents except the test sample), and A sample is the absorbance of test samples values, which represented the concentration of *Amaranthus viridis* that caused 50% inhibition, were calculated from the plot of percentage against concentration. The radical scavenging activity by hydrogen or electron donation is a marker of antioxidant activity. These activities could be attributed to hydrophilic antioxidants (phenolics) and lipophilic (carotenoids) antiradical substances. In addition, RSC (radical scavenging capacity) activity decreased in the AE extracts. *Aminetal* obtained similar results. Samarth *et al.* reported that In our work, *A. cruentus* WE showed higher DPPH-radical scavenging activity . Antioxidant activities of extracts using the DPPH assay correlated well with total flavonoids. The flavonoid antioxidant properties are linked to their ability to bind metallic ions, which can be involved in free-radical genesis. Radical chain reactions are broken by flavonoid hydrogen donors and also by their capacity to regenerate  $\alpha$ -tocopherol. Flavonoids possess a remarkable spectrum of biochemical and pharmacological activities that have been attributed, in part, to their antioxidant activities.

## RESULT-

### Phytochemical Screening:

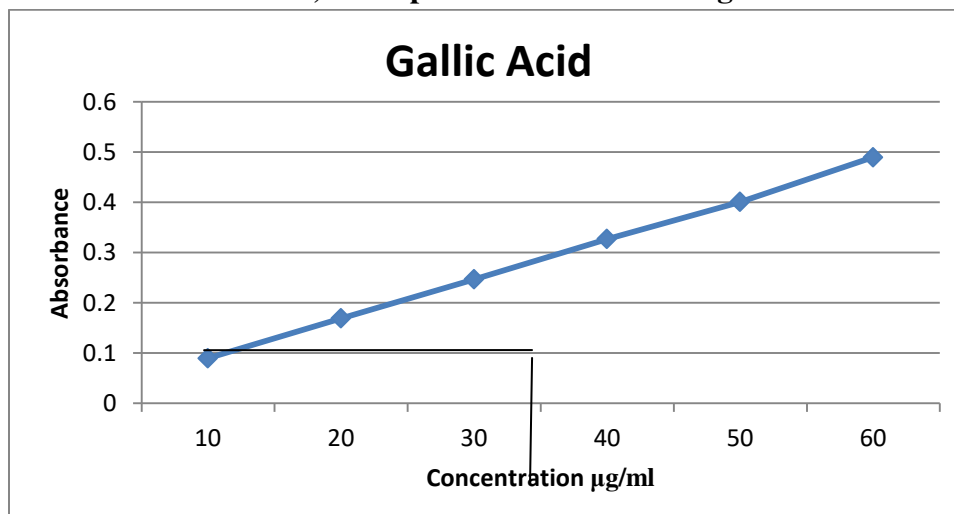
Test	Methyl Extract	Aqueous Extract
Polyphenol & Tannis	+	+
Flavonoids	+	-
Anthracenosides	-	-
Steroids	-	-
Caretinoids	+	-
Saponins	-	+
Alkaloids	-	-

Analyte	Content
Total Phenolic	2.81 TO 3.81 mg GAE \100 mg extract
Total Flavonoide	18.4 to 5.41 mg CE \100 mg extract
Alkaloid	10.45 to 15.95%

**Gallic acid-**

Sr.no.	Conc.	Absorbance
1.	10	0.09
2.	20	0.169
3.	30	0.247
4.	40	0.327
5.	50	0.401
6.	60	0.490

**Test abs- 0.27, Total phenolic content-0.003gm/100ml**

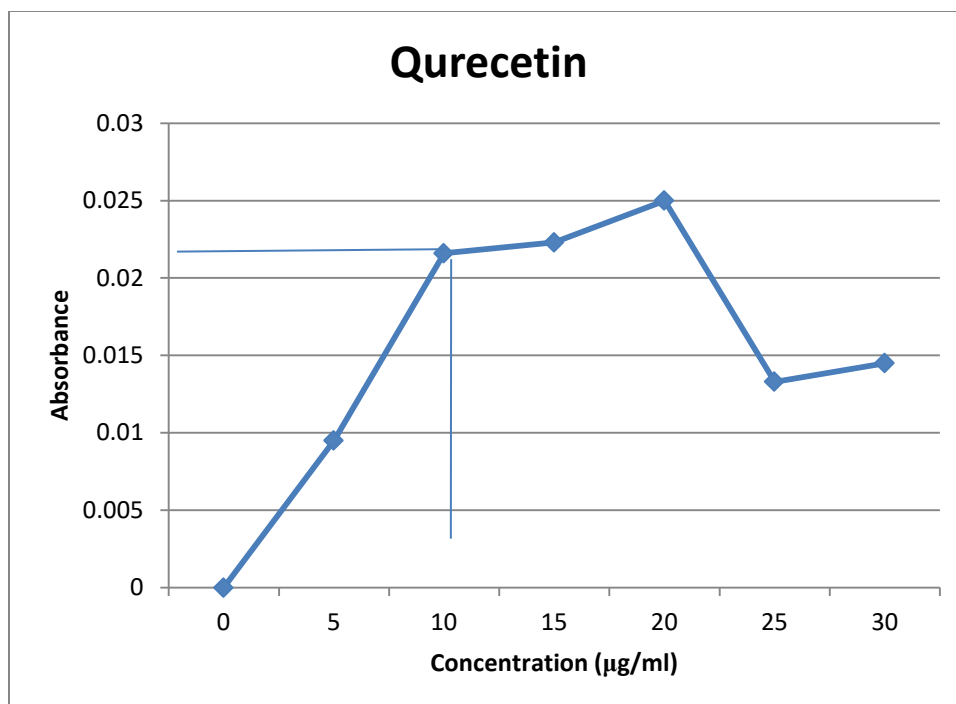


**Quercetin-**

Sr.no.	Conc.	Absorbance
1.	5	0.0095
2.	10	0.0216
3.	15	0.0223
4.	20	0.0256
5.	25	0.0133
6.	30	0.0145

**Test abs-0.023, Total phenolic content-0.001gm/100ml**





**DPPH-**

**1) Ascorbic Acid-**

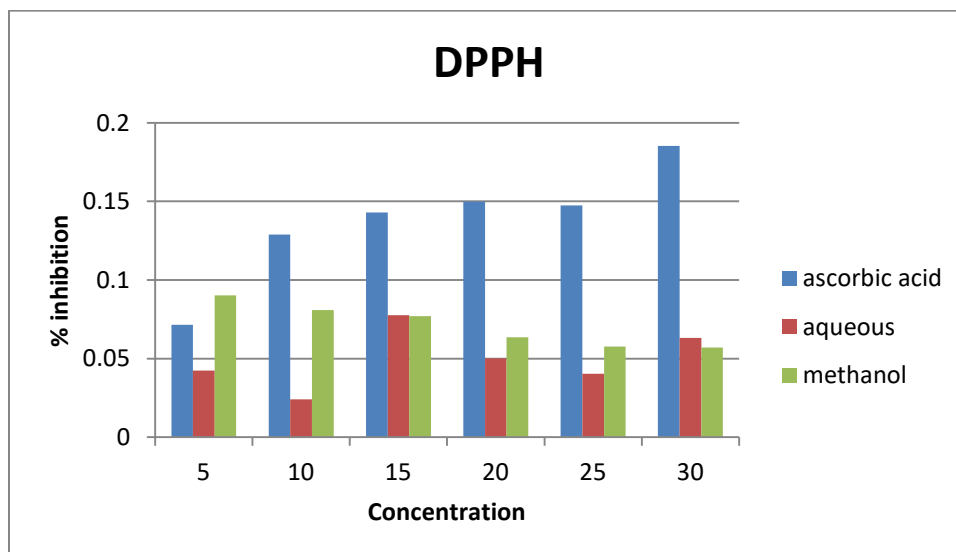
Sr.no.	Conc.	Absorbance
1	5	0.0714
2	10	0.1288
3	15	0.1429
4	20	0.1499
5	25	0.1475
6	30	0.1852

2) Aqueous

Sr.no.	Conc.	Absorbance
1.	5	0.0423
2.	10	0.0240
3.	15	0.0776
4.	20	0.0502
5.	25	0.0404
6.	30	0.0632

Methanol-

Sr.no.	Conc.	Absorbance
1.	5	0.0903
2.	10	0.0809
3.	15	0.0769
4.	20	0.0635
5.	25	0.0576
6.	30	0.0570



## CONCLUSION –

The present study has contributed to understanding the activity of *Amaranthus hypochondriacus* grains as an excellent source of phytochemicals like phenolics, flavonoids, alkaloids and saponins. The various experimental analyses carried out on *Amaranthus hypochondriacus* grains revealed its potential as a good nutraceutical, thereby, offering various health benefits. The methanolic extract of the sample exhibited high antioxidant activity.

## REFERENCE-

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