Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2017; 6(4): 103-105
Received: 06-05-2017
Accepted: 05-06-2017

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# Study the antioxidant and *In vitro* Anti-inflammatory activity by membrane stabilization method of *Amaranthus gangeticus* leaf extract

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**Abstract**
Since the ancient time nature provides several herbal phyto-chemicals for the beneficial of human. *Amaranthus gangeticus* of the family amaranthaceae, is such a leafy edible vegetable widely used in eastern zone of India. In the current study, qualitative screening of antioxidant property and membrane stabilization property was checked in aqueous and alcoholic extract of *Amaranthus gangeticus*. Three different concentration 50, 75, 100 microgram/ml ethanolic and aqueous extract was taken for membrane stabilization method using 50 microgram/ml sodium diclofenec as a reference drug and only aqueous extract of the same concentration was used in total antioxdidant determination method using ascorbic acid as reference. The study revealed that *Amaranthus gangeticus* have several significant membrane stabilization and antioxidant property. Presence of delicate chemical balance of the whole plant, or mixtures of plants, not one particular active ingredient, may help to scavenge the reactive oxygen, potent membrane destabilizing agent in human. So, in future it could be possible to develop a new phyto derived drug for membrane destabilization related disease in human.

**Keywords:** *Amaranthus gangeticus*; Membrane stabilization; Antioxidant property; Whole plant extract

## Introduction
Use of different herbs and plants as an alternative and complementary therapy is a category of medicine that includes a variety of treatment approaches that fall outside the realm of conventional medicine. These traditional herbal medicines, often known as a good source of neutraceuticals, possess various beneficial effects on human health. In recent years an increasing amount of research regarding neutraceuticals is being done to establish the safety and efficacy of these therapies, though compared with mainstream medical therapies researches are still meagre.

As growing interest in these nutraceuticals or functional foods is leading to detailed research, it became absolute necessary to test the plants involved thoroughly for their different medicinal properties as neutraceuticals are natural compounds with bioactive properties having health promoting, disease preventing or curative properties [1]. It has been found that most of the observed therapeutic effects of plants are linked to their potent antioxidant activity. It is also said that this kind of healing activity could be the traditional basis of plants used in Ayurveda

In human oxidative stress has been identified as the basic cause of development and progression of several diseases. Oxidative stress is a condition where reactive oxygen species (ROS) being produced more than the amount of antioxidant being generated in defense. Endogenous oxidants like hydrogen peroxide, superoxide anion and hydroxyl radicals are the major inducer of oxidative stress. About 5% of inhaled oxygen was converted to these kinds of reactive oxygen species after normal cellular metabolism [2]. Lack of proper normal endogenous antioxidant production and unavailability of counter-oxidative species disrupt the body homeostasis and may lead to oxidative stress condition which causes serious damage to the DNA, proteins and other sub cellular components. It has been reported that severe to mild oxidative stress may cause disease like cancer, hypertension, arthritis, arteriosclerosis etc. Scavenging the reactive superoxide seems to be the way to maintenance of body equilibrium.

As estimated 12,000 of the world's plants are edible among which the importance of leafy vegetables are well known as they are known to give health protection due to the presence of important secondary metabolites [3].

*Amaranthus gangeticus* of the family amaranthaceous, is such a leafy vegetable widely used in eastern zone of India. It is an invariable part of traditional Bengali cuisine and has huge medicinal property as some native practitioner used this as haepatoprotective agent against Jaundice [4]. Literature study revealed that other *Amaranthus* species like *A. tricolor, A. viridis*

have potent antioxidant and antiinflammatory activity and *A. gangeticus* have potent hepatoprotective and DPPH free radical scavenging activity [4, 5, 6]. Keeping the traditional folk or native knowledge and literature review in concern, the selected plant was used to determine its total anti-oxidant property by phospho-molybdenum method and *in vitro* Anti-inflammatory property by HRBC membrane stabilization method. In the current study both aqueous and ethanolic extract of shed dried *Amaranthus gangeticus* leaves were used for *in vitro* membrane stabilization test and only aqueous extract was used for antioxidant test.

Many modern drugs are made from plants. But herbalists don't extract plant substances in the way the drug industry does. Herbalists believe that the remedy works due to the delicate chemical balance of the whole plant, or mixtures of plants, not one particular active ingredient [7]. Believing in this principle we used the whole extract for the present study.

## Materials and Methods

### Collection of Sample

Fresh leaves of *Amaranthus gangeticus* were collected from a local market during the month of March and the plant was Trully identified by Prof. and Head. Department of Biotechnology, IGE.

### Preparation of Extract

Fresh leaves were washed twice through running tap water followed by distilled water and finally air dried. Leaves were blended to fine powder after thorough drying. The shade dried powder of leaves was stored in room temperature for future use. The dried powdered 1 gm leaves were taken in two different pre-labeled conical flasks and 40 ml of double distilled de-ionized water and ethanol were added separately. The mixtures were kept in the BOD shaker incubator at 30ºC temperature in 120 rpm overnight. Both the mixture was filtered through Whatman filter no. 1 after 24 hrs. During the membrane stabilization assay every time freshly prepared aqueous and alcoholic extracts were used.

### *In vitro* Membrane stabilization assay

To study the anti-inflammatory activity, the HRBC membrane stabilization method was adopted (Gandhisan 1991) [8]. Fresh blood was collected from healthy donors without having the history of NSAIDS administration for at least two weeks prior to the experiment. The equal volume of sterilized Alsever solution and blood were mixed, the mixture was centrifuged at 3000 rpm and packed cell were washed twice with isosaline. The washed packed cell was made as a 10% (v/v) suspension with isosaline to make a HRBC suspension. The assay mixture contained 1ml PBS (pH 7.4), 2ml of hyposaline (0.36% KCL), 0.5ml HRBC suspension and 1 ml of Test solution.

Sodium diclofenec was used as reference drug and distilled water was used as control. All the assay mixtures were incubated at 37ºC in an incubator for 30 minutes and after that centrifuged at 1000 rpm for 2 minutes. The supernatant containing haemoglobin was estimated using spectrophotometer at 560 nm.

The percentage inhibition of membrane stabilization was calculated by using the following formula

% inhibition = $100 \times [OD_1\text{-}OD_2/OD_1]$

Where, $OD_1$ = absorbance of Control, $OD_2$ = absorbance of test sample.

### Total antioxidant activity

The total antioxidant activity of *Amaranthus gangeticus* leaf extract were evaluated according to Prieto *et al*., 1999 [9]. 0.3ml of various concentration of aqueous leaf extract (50, 75, 100 µg/ml) were mixed with 3ml assay mixture which contain 4mmol/L ammonium molybdate, 0.6mol/L sulphuric acid and 28mmol/L sodium phosphate. Then the assay mixture with test sample was incubated at 95ºC for 90 min in water-bath. After cooling to 25ºC, absorbance of the final solution was measured at 695 nm wave length in spectrophotometer. Vehicle (Distilled water) was used as blank and ascorbic acid as positive control.

The percentage of antioxidation property was calculated by using the following formula

%= [(OD sample-OD blank) / (OD ascorbic acid-OD blank)] X100

## Result

In the present study, various concentrations of both aqueous and ethyl alcoholic *Amaranthus gangeticus* leaf extracts were used for the membrane stabilization assay whereas only aqueous extract was used for antioxidant assay. The results obtained were depicted in Table-1 and Table-2.

Concentration of reference drug and the experimental samples ware also mentioned in the tables.

**Table 1**: *In vitro* membrane stabilization activity of aqueous and ethanolic extract of *Amaranthus gangeticus* leaves.

| Treatment | Concentration(µg/ml) | % Inhibition |
|---|---|---|
| Control | ---------- | ---------- |
| Ethyle Alcohol Extract | 50 | 42.9 |
| | 75 | 44.8 |
| | 100 | 52.1 |
| Aqueous Extract | 50 | 24.4 |
| | 75 | 34.5 |
| | 100 | 39.6 |
| Sodium Diclofenec | 50 | 62.99 |

**Table 2:** Antioxidation activity of aqueous extract of *Amaranthus gangeticus* leaves

| Treatment | Concentration(µg/ml) | Antioxidation (%) |
|---|---|---|
| Aqueous Extract | 25 | 12.60 |
| | 50 | 20.40 |
| | 75 | 28.57 |
| | 100 | 77.90 |

## Discussion

It is a well-known fact that hydrolytic enzymes released during inflammation generate different disorders. The extra cellular activity of these enzymes is said to acute or chronic inflammation [10]. Both the aqueous and alcoholic extract of the tested plant in the present study showed moderate to high membrane stabilization property in concentration dependent manner. But, when compared with aqueous extract, ethanolic extract of the leaves showed higher membrane stabilization activity. The erythrocyte membrane is analogous to the lysosomal membrane and the stabilization of red blood cell membranes implies that the test extract may well as stabilize lysosomal membranes. Stabilization of lysosomal membrane has immense importance in limiting the inflammatory responses by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which in turn induces further tissue inflammation and damage up on extra cellular release. Most of the non-

steroidal drugs act as an anti-inflammatory agent either by inhibiting those hydrolytic enzymes or by membrane stabilization process. Since HRBC membrane are similar to Lysosomal membrane components the prevention of HRBC membrane lysis is taken as a measure of anti-inflammatory activity. In this current study as both the aqueous and alcoholic extract of *Amaranthus gangeticusI* leaves exhibit erythrocytic membrane stabilization activity, it may be also used for lysosomal membrane stabilization which in turn can act as a potential anti-inflammatory agent.

In the present study the total antioxidant property of the aqueous extract of *Amaranthus gangeticus* leaves was tested and it confirmed the ability of antioxidation as the highest concentration 100µg/ml shows 77.99% antioxidation property as compared to standard ascorbic acid. So the aqueous leaf extract may be used as a scavenging agent of reactive oxygen species, the fundamental causative agent of oxidative stress. Oxidative stress tends to be the mother of other disorder like ulcer, cancer, arthritis, hypertension and so on. If by means it will be successfully tackled by any natural, herbal, and zero side effect alternative substance, it could be helpful in countering many other follow up diseases. This reactive oxygen like direct oxidizing superoxide [2] and indirectly as with hydrogen peroxide ($H_2O_2$) and hydroxyl radicals (•OH) formed from $O_2^-$, harms the surrounding tissue by lipid peroxidation which leads membrane destruction and provokes inflammatory response by the production of mediators and chemotactic factors. So scavenging these super oxides due to the antioxidation property of the *Amaranthus gangeticus* may also help to increase the anti-inflammatory effects of the plants.

## Conclusion

It has been found from the present study, that, the ethanolic and aqueous extract of *Amaranthus gangeticus* was able to stabilize erythrocytic membrane *in vitro* and was comparable to the anti-inflammatory compound sodium diclofenec. The aqueous extract of *Amaranthus gangeticus* also exhibit potent role in scavenging free radicals, which plays a major role in different metabolic pathways. The anti-inflammatory and antioxidant effect of this plant should be further evaluated to confirm the bioactive compounds responsible for these activities in pursuit of newer phytotherapeutics against inflammatory diseases and many oxidative stress related disease

**Funding statement:** "This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All the research work done by the affiliated institutions' funding.

**Competing Interests Statement:** The authors declare that they have no competing interests.

**Contributor ship Statement**: Dr. M.J Mukhopadhyay helped with the concept and discussion. Mr. Puspal De and Mr. Subhradeep Sarkar performed all the experiments and analysis part in the laboratory. All authors read the manuscript and agreed for publication.

**Data Sharing Statement:** We cannot share any unpublished data with other laboratory or person.

**Ethics Approval:** Present study was approved by the institutional ethical committee.

**Acknowledgement**

All authors acknowledge the Director and Vice Principal of Institute of Genetic Engineerimg for funding and affiliation. We are also thankful to other laboratory members and other associated person of IGE for their enthusiastic participation.

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