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ANTIOXIDANT STUDIES, ERYTHROCYTE MEMBRANE STABILIZATION AND THROMBOLYTIC POTENTIAL OF DIALLYL DISULPHIDE

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Keywords:

DADS- Diallyl Disulphide, DPPH- 1, 1-diphenyl-2-picryl hydrazyl, TPTZ- 2, 4, 6-tripyridyl–S-triazine, FRAP- Ferric Reducing Antioxidant Potential, Erythrocyte Membrane Stabilization, Thrombolytic Potential, Reactive Oxygen Species

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ABSTRACT: Diallyl Disulphide is an essential component of garlic which has numerous medicinal applications. Thus it was evaluated for its free radical scavenging potential. The compound was assayed for its ability to stabilize the erythrocyte membrane by osmotic fragility test and heat induced hemolysis assay. The thrombolytic potential was evaluated by the ability of the compound to dissolve the clot formed. The antioxidant studies were assessed by DPPH radical scavenging assay, ferric reducing antioxidant potential, Superoxide radical scavenging potential and Nitric oxide radical scavenging potential. The total antioxidant capacity was also estimated and the values were expressed in terms of Ascorbic acid equivalents. The results show a poor free radical scavenging potential and antioxidant capacity. However it has shown a moderate percentage in dissolution of clot implicating in thrombolytic potential. The compound has also shown a higher erythrocyte membrane stabilization by reducing the percentage of hemolysis in heat induced hemolytic assay than Ascorbic Acid. The results suggest that Diallyl Disulphide is a poor antioxidant but a potential anti-hemolytic in *In-vitro* studies.

INTRODUCTION: Diallyl Disulphide is an organosulphur compound derived form allicin, natural compound found in garlic and related plants [1, 2]. Diallyl Disulphide has many physiological effects, many that are cardio- and neuroprotective. Diallyl Disulphide is also a known antiatherosclerotic, antibacterial and antimutagenic [3, 4]. These effects are due to, atleast in part because Diallyl Disuphide is converted, in the presence of thiols, to the gaseous mediator Hydrogen Sulphide[5].

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Thus, Diallyl Disulphide serves as a thiol-dependent Hydrogen Sulphide donor in biological systems. There is limited literature available about the ability of Diallyl Disulphide in stabilizing the erythrocyte membrane at elevated temperatures. Previously aged garlic extracts have been shown to be a potential therapeutic source for sickle cell anemia [6]. The Anticancer property of garlic has been attributed to the presence of Diallyl Disulphide and other organosulfur compounds [7]. Thus, Diallyl disulphide could be a good source of therapeutic and other biomedical applications. The present study attempts to evaluate the antioxidant, erythrocyte membrane stabilization and thrombolytic potential of Diallyl Disulphide.

MATERIALS AND METHODS:

The entire experiment was carried out at the Nitte University center for Science Education and

Research (NUCSER), Nitte University, Mangalore, India.

The Diallyl Disulphide (>75% pure; GC graded) was obtained from TCI chemicals, Japan. The DPPH, TPTZ were purchased from Himedia pvt. Ltd., Mumbai. The Antioxidant studies were carried out by DPPH radical scavenging assay, Nitric oxide radical scavenging assay and Superoxide radical scavenging assay. The ferric reducing antioxidant power assay was done by TPTZ.

A.

B.

DPPH Assay:

The method of [8] was followed. The free radical scavenging activity of the various extracts of *Carica papaya* (Linn.) were measured with formation of stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical using a spectrophotometer at 517nm. The percentage inhibition value was calculated using the formula,

$$\frac{\text{Absorbance of Control- Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

FRAP-TPTZ Assay:

The method of [9] was used. The Ferric Reducing Antioxidant Power was determined using 2, 4, 6-tripyridyl–S-triazine (TPTZ) in a spectrophotometer at 595nm. The results were expressed in terms of EC_{50}, the concentration at a particular absorbance at which it is exactly half of the absorbance of the control.

Nitric Oxide Radical Scavenging Assay:

The method of [10] was followed to evaluate the Nitric Oxide radical scavenging property. The Nitric Oxide Radical Scavenging Activity was measured by the formation of nitrite from sodium nitroprusside using Griess reagent (1% Sulphanilamide in 2.5% ortho-phosphoric acid, 0.1% Naphthyl ethylenediamine dihydrochloride) spectrophotometrically at 540nm.

Superoxide Radical Scavenging Assay:

The method of [11] was followed with slight modification. The superoxide Radical generated by the photo-oxidation of methionine-riboflavin and their scavenging property was determined spectrophotometrically at 560nm using Nitroblue tetrazolium (NBT) as the chromogenic substrate.

Total Antioxidant Assay:

The estimation of total antioxidants was done by the method of [12]. The formation of blue- green phosphomolybdenum complex was measured at 695nm.

Estimation of Total Phenolic Content:

The method of [13] was followed to estimate the total phenolic content using Folin- Ciocalteau reagent. The absorbance was measured at 760nm.

***In-vitro* Erythrocyte Membrane Stabilization and Thrombolytic Potential:**

Heat Induced Hemolysis assay:

The method of [14] was followed. The red blood cell suspension was pre-incubated with different extracts of *Carica papaya* (Linn.) for 20minutes at 55^0C in a water bath. Then the absorbance of the suspension was measured at 540nm. Aspirin was used as positive control.

Osmotic Fragility of Erythrocytes:

The method of [15] was followed with slight modifications. The erythrocyte suspension with the extracts was added to serially diluted saline from 0.9% to 0.48% and distilled water. The absorbance of the suspension was measured at 540nm. The absorbance at 0.44% was taken against the absorbance with erythrocyte suspension in distilled water as control.

Thrombolytic Potential:

The thrombolytic potential was evaluated by the method of [16]. The extracts were incubated with pre-weighed clot for 90 minutes at 37^0C. The clots were centrifuged, the supernatant discarded and clot weighed again. The percentage of clot dissolved was calculated.

RESULTS:

***In vitro* Antioxidant study on Diallyl Disulphide:**

- **DPPH assay:**

TABLE 1 SHOWING THE DPPH RADICAL SCAVENGING POTENTIAL OF DIALLYL DISULPHIDE COMPARED WITH STANDARD ASCORBIC ACID

Concentration in mg	% Inhibition by DADS	% Inhibition by Ascorbic Acid
10	0.87	35.53
20	3.79	41.40
30	4.22	47.58
40	8.74	55.34

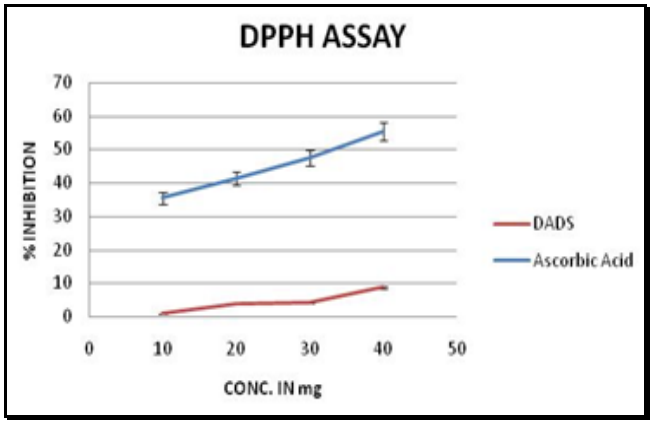

FIG.1 SHOWING THE COMPARISON BETWEEN DPPH RADICAL SCAVENGING POTENTIAL OF DIALLYL DISULPHIDE AND ASCORBIC ACID

- **FRAP-TPTZ assay:**
  The Ferric Reducing Power of Diallyl Disulphide was found to be 566.83±231.5mg compared to that of Ascorbic Acid standard with a FRAP value of 25.34±1.55 mg.

- **Superoxide radical scavenging assay:**

TABLE 2 SHOWING THE SUPEROXIDE RADICAL SCAVENGING ACTIVITY OF DIALLYL DISULPHIDE COMPARED WITH STANDARD ASCORBIC ACID

Concentration in µg	% Scavenging Activity of DADS	% Scavenging Activity of Ascorbic Acid
200	2.5	10
400	3.6	13.6
600	7.7	16

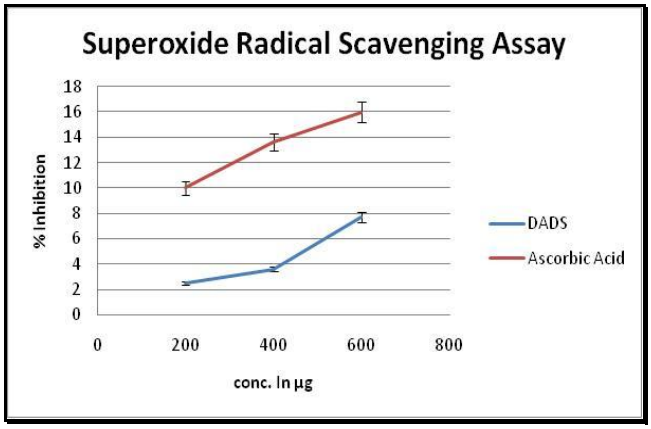

FIG. 2: SUPEROXIDE RADICAL SCAVENGING ASSAY OF DIALLYL DISULPHIDE AND ASCORBIC ACID

- **Nitric Oxide radical scavenging assay:**
  The Nitric oxide scavenging potential of Diallyl Disulphide was found to be 5.30% compared to that of Ascorbic Acid with 27% of Scavenging potential.

- **Estimation of Total Antioxidants:**
  The total antioxidants present in Diallyl Disulphide were about 41.33±13.32 µM Ascorbic Acid Equivalents.

***In-vitro* Erythrocyte Membrane Stabilization and Thrombolytic Potential:**

Heat Induced Hemolysis assay: The results of heat induced hemolytic assay are shown in **Figure 3**. A concentration of 100mg of the compounds was selected for the experiment.

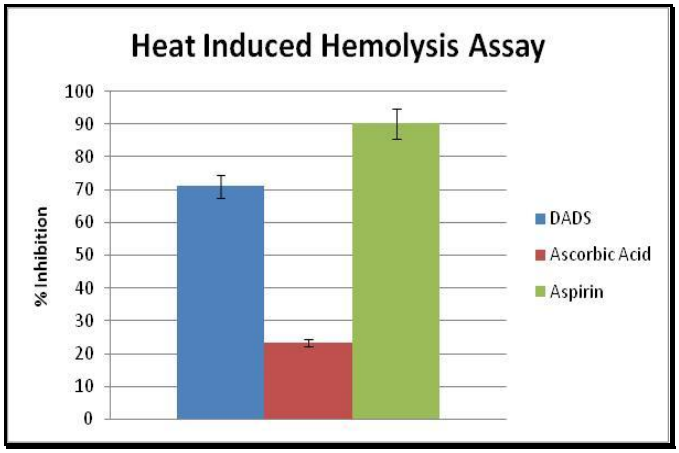

FIG. 3: HEAT INDUCED HEMOLYSIS ASSAY OF DIALLYL DISULPHIDE AND ASCORBIC ACID

Osmotic Fragility of Erythrocytes: Diallyl Disulphide showed 0% inhibition in hypotonicity induced hemolysis as compared to that of Ascorbic Acid with 29.6% inhibition of hemolysis.

Thrombolytic Potential: The thrombolytic potential of Diallyl Disulphide is displayed in **Figure 4**.

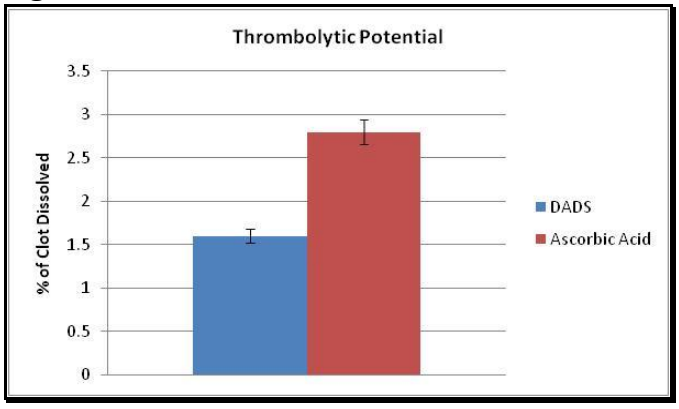

FIG. 4: PERCENTAGE OF CLOT DISSOLVED BY DIALLYL DISULPHIDE AND ASCORBIC ACID

DISCUSSION: The antioxidant results from DPPH, TPTZ indicate a poor hydrogen donating ability and thus diminished ability to reduce the free radicals. The ability to donate electrons and scavenge the free radicals by Diallyl Disulphide is not significant compared to that of Ascorbic Acid which shows a five time higher capacity. Its thrombolytic potential when compared to streptokinase [16] was not significant (p value >0.05). But Diallyl Disulphide has shown a higher erythrocyte membrane stabilization by reducing the percentage of hemolysis in heat induced hemolytic assay than Ascorbic Acid. Thus Diallyl disulphide, a component of garlic oil might also have an important role in inhibiting the hemolysis and enhancing the membrane integrity at elevated temperatures.

CONCLUSIONS: The results suggest that Diallyl Disulphide is a poor antioxidant but a potential anti-hemolytic in *In-vitro* studies. Hence further *In-vivo* studies can be undertaken to evaluate the mechanism of the various physiological changes caused by Diallyl Disulphide.

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