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# INHIBITION OF REACTIVE OXYGEN SPECIES AND LIPID PEROXIDATION BY BOTH AQUEOUS AND ETHANOLIC EXTRACTS OF *FRAGARIA ANANASSA* USING *IN VITRO* ASSAY

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**ABSTRACT**

Lipid peroxidation is take place in the human body during the free radical reaction due to reactive oxygen species generation. During the reaction it releases the degraded components like malonyl dialdehyde (MDA) components which forms a complex with thiobarbutric acid (TBA) it yield a pink colour. Inhibition of peroxidation is determined by low level of MDA by reduced level of pink colour formation. It was assayed by using thiobarbutric acid as detecting agent.

**Keywords**: Thiobarbutric acid, Malonyl dialdehyde, Peroxidation, Detecting agent.

## INTRODUCTION

A free radical is an agent which consists of unpaired electron in outer shell [1]. Generally the free radical is unstable due to its lone pair of electrons. So that they steal the electron from other molecule to become stable and paired [2]. According to Free Radical Theory of Aging(FRTA) the organisms undergone age due to deposition of free radical agent [3]. Free radical mainly involve in chain reaction by cross linking the base pair of DNA strand, the chain reaction involves in three steps process Initiation, Propagation, Termination [4]. Superoxide and nitric oxide are the main free radicals involved in the process of aging [5] Free radical chain reaction are mainly responsible for aging and other chronic diseases [6].

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidant such as thiols or ascorbic acid terminates this chain reaction and involve in reducing and preventing damage from free radical reaction. Herbal plants consist of consist of antioxidant property. The compound which has antioxidant property also contains anti-inflammatory, anti-tumour, antimutagenic, anticarinogenic, antimicrobial activites [7].

Strawberry is an berry fruit that has chemical constituents like ellagitannin, ellagic acid, flavonoids like anthocyanins & phenolic acids such as hydroxybenzoic acid, hydroxycinnamicacid [8-10]. Commonly the tocopherol, carotenoids, ascorbic acid& phenolic acids are essential contents for antioxidants property [11]. Strawberry fruit has more abundance level of antioxidant effect [2, 13]. Anthocyanins were present in strawberry fruit which is more comprises of total antioxidant capacity [14].

Thio-barbutric acid method is use to determine the degradation products of fats in lipid peroxidation, which is shortly called as Thio-barbutric Acid Reactive Substance (TBARS). In this assay thiobarbutric acid is use to identify the reactive oxygen species though it has short half-life [15]. In TBARS method, the usage of thiobarbutric acid reagent use to identify the malondialdehyde which was generated during lipid peroxidation [16]. By this invitro method of TBARS use to designate the generation of free radical generation.

Ageing is the process of accumulation of free radical substance that leads to DNA oxidative damage and also programmed ageing led to apoptosis which leads to major risk factor [17]. Antioxidant is the agent use to eradicate the generation of free radical(reactive oxygen species) and prevent ageing.

## MATERIALS AND METHOD

### Chemical and reagents

Trichloroacetic acid AR, Thiobarbutric acid AR, Sodium Deodecyl Sulphate AR, Acetic acid AR all the chemicals were obtained from Loba chemicals Ltd. Ethanol AR, Butanol AR, Distilled water the solvent are used in analytical grades and obtained from Merck, Mumbai, India. Centrifugee were obtained from advanced technocracy Inc. Grain Market, Colorimeter were obtained

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from Systonic Industrial area.

**Sample Collection**

The fruits of strawberry were collected from local market of different region in Tamilnadu. Collected leaf were washed and dried at room temperature for 3 days and finely powdered in grinding machine for ease extraction.

**Preparation of Aqueous extracts**

The powdered materials of about 350g in 900ml of aqueous solvents in a large container and was regularly shaken for 3 days at room temperature. It was then filtered and filtrate was concentrated.

**Preparation of Ethanolic extracts**

The powdered materials of about 350g in 900ml of ethanol solvents in a large container and was regularly shaken for 3 days at room temperature. It was then filtered and filtrate was concentrated.

**Lipid Peroxidation Inhibition Assay**

In lipid peroxidation, Malonyl dialdehyde is the substance which is released during free radical generation and its combines with thiobarbutric acid and produces pink color which was measure in colorimeter. It is mainly use to determine the thiobarbutric reactive species in solution

$$\text{Lipid peroxidation inhibition assay} = \frac{Blank - sample}{sample} \text{ X } 100$$

**Experimental design**

0.5ml egg yolk homogenate were prepared and 0.1ml of sample extract was mixed along with 0.05ml of ferrous sulphate incubate for 30mins. Then add 20% of acetic acid, 0.8% of thiobarbutric acid in 1.1% of sodium deodecyl sulphate, 20% of trichloroacetic acid is added. Vortexes and heat for 60mins. Butanol is used as solvents and centrifuge at 3000rpm for 10mins. Measure the absorbance at 532nm.

**RESULT**

In Figure 3 shows that the different extracts of aqueous and ethanolic, which are kept for measuring the absorbance at 532nm. In this the test tube shows difference in color absorption indicates the inhibition the lipid peroxidation process.

In figure 3 shows that the percentage inhibition of lipid peroxidation by graphical representation which indicates that the suppression of free radical generation of aqueous and ethanolic extracts. From this representation the aqueous extract has inhibiting the peroxidation at 800mg and ethanolic extract at 200mg. By measuring the color absorbance at 532nm the absorption of two extracts were plotted in graph.

**Fig 1. Strawberry fruit**

**Fig 2. Aqueous and ethanolic extract**

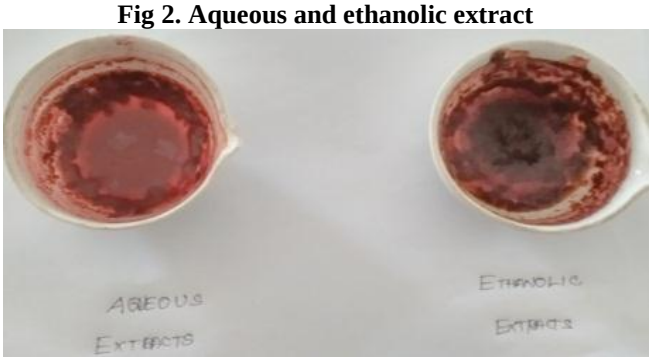



**Fig 3. During the colour absorbance of aqueous and ethanolic extract**

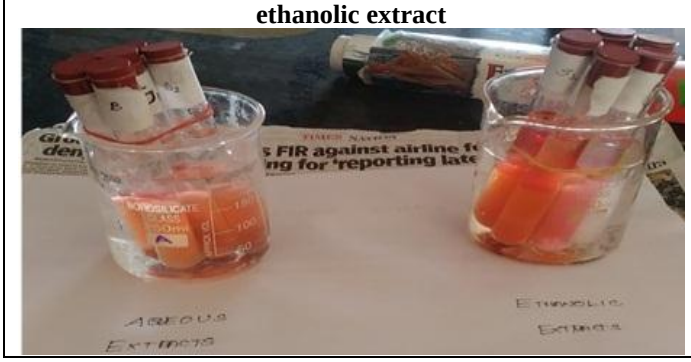
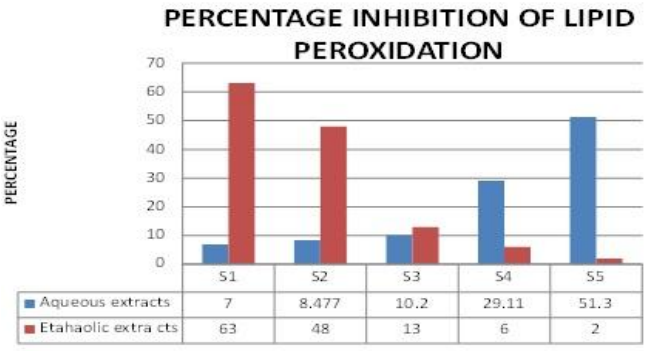


**Fig 4. Percentage of lipid peroxidation using strawberry**




**DISCUSSION**

Free radicals react and produces oxidative stress in our body leads to diseases, premature aging, diabetes, inflammations, asthma, tissues, heart diseases and other chronic disorders.[19]. Malonyl dialdehyde was the end product of reactive oxygen species during free radical reaction, when it combines with TBA it produces the colour [19]. The sample was treated against free radical reaction by made in an aqueous and ethanolic extracts. The aqueous extract shows the lipid peroxidation inhibition capacity better than ethanolic extract.

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**CONFLICT OF INTEREST**

No interest

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