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IN VITRO ANTIOXIDANT ACTIVITIES OF CHLOROFORM EXTRACT OF *ANNONA SQUAMOSA*.

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Abstract:

The antioxidant activity of the plant extracts was evaluated by DPPH radical scavenging mechanism. DPPH is a free radical compound that has widely been used to test the free radical scavenging abilities of various types of plant extracts. The antioxidant activities of chloroform extract of Annona squamosa aerial parts were analysed. Plants are rich in a variety of phytochemicals including tannins, terpenoids, alkaloids, and flavonoids which have been found in vitro to have antimicrobial properties. Although the mechanism of action and efficacy of these herbal extracts in most cases is still needed to be validated scientifically, these preparations mediate important host responses. Therefore it is of interest to investigate the in vitro antioxidant efficacy of chloroform extract of Annona squamosa aerial parts.The results of the present study reveals that the antioxidant potential of A.squamosa.

Key words: *Annona squamosa, Free radicals, Herbal plants, Human diseases, Terpenoids.*

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INTRODUCTION:

The search for phytochemicals with potent antioxidant continues to be of great importance in the search for remedies against free radical-mediated diseases, prevention of oxidative reactions in foods, protection against DNA damage and carcinogenesis, and possible substances with wide range of pharmacological activities such as anti-inflammatory, anti-bacterial, and anti-fungal properties. [1]. Natural products are defined as natural sources-derived substances having biological activities. Natural products have long been implemented as alternative health care treatment and in discovery of modern drugs [2]. A major focus of natural product chemistry has been toward drug design and discovery. Medicinal plants refer to the class of plants applied for therapy or to possess pharmacological actions for human and animal [3]. Further investigation on isolation and characterization of bioactive compounds derived from natural extracts is progress in our lab. *Annona squamosa* is a small, semi-deciduous tree, 3-7 m in height, with a broad, open crown or irregularly spreading branches; bark light brown with visible leaf scars and smoothish to slightly fissured into plates; inner bark light yellow and slightly bitter; twigs become brown with light brown dots [4-7].

MATERIALS AND METHODS:

Collection of medicinal plants

The Indian medicinal plant *Annona squamosa* collected from the medicinal garden, Chennai, India. The parts of the plants were authenticated by the botanist.

Plant Materials

The aerial parts of Chloroform extract of *Annona squamosa* were used for this study.

Preparation of Plant extracts

The extraction of the plant material was carried out using known standard procedures. The plant materials were dried in shade and powdered in a mechanical grinder. The powder (25.0 g) of the plant materials were initially defatted with petroleum ether (60-80°C), followed by 900 ml of hydroalcohol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The hydroalcoholic extract yields a dark greenish solid residue weighing 5.750 g (23.0% w/w). More yields of extracts were collected by this method of extractions. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml. The extract was preserved at 2- to 4°C.

Chemicals and Reagents

All chemicals were used for this project were purchased from M/s. Sigma Chemicals, USA.

Determination of Antioxidant activity (DPPH free radical scavenging activity)

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity [4]. Ethanolic solution of DPPH (0.05 mM) (300 l) was added to 40: l of extract solution with different concentrations (0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation. Percent (%) inhibition of DPPH activity = [(AB – AA) / AB] x 100 Where AA and AB are the absorbance values of the test and of the blank sample, respectively [8].

RESULTS AND DISCUSSION:

Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter. Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. The free radicals (oxidants) are species with very short half-life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. In general, the reactive oxygen species circulating in the body tend to react with the electron of other molecules in the body and these also effect various enzyme systems and cause damage which may further contribute to conditions such as cancer, ischemia, aging, adult respiratory distress syndromes, rheumatoid arthritis etc.

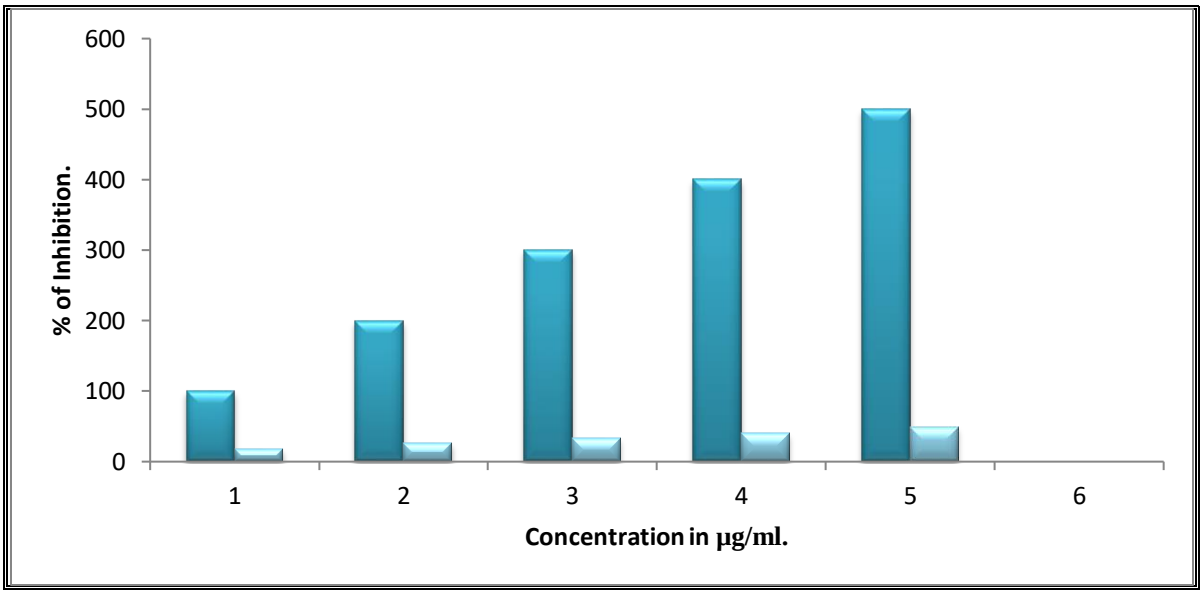

Fig. 1: shows the antioxidant activity of standard BHT

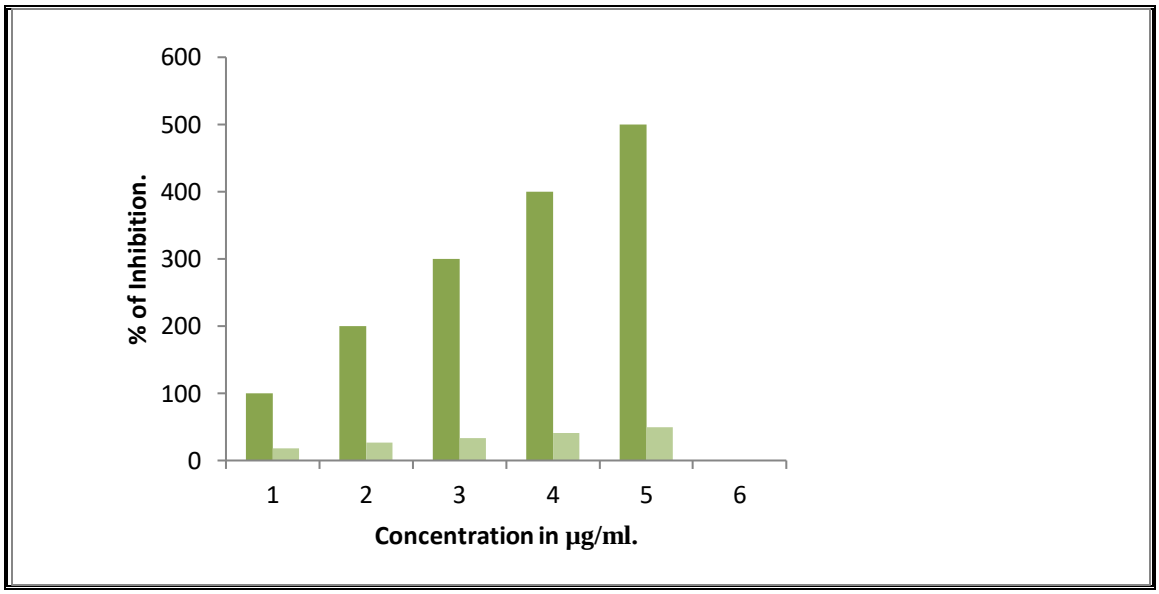

Fig. 2: shows the antioxidant activity of chloroform extract of *Annona squamosa.*

A plant-based diet protects against chronic oxidative stress-related diseases. Dietary plants contain variable chemical families and amounts of antioxidants. It has been hypothesized that plant antioxidants may contribute to the beneficial health effects of dietary plants. The screening and characterization of antioxidants derived from natural sources has gained much attention and efforts have been put into identifying compounds as suitable antioxidants to replace synthetic ones [9]. The search for phytochemicals with potent antioxidant continues to be of great importance in the search for remedies against free radical-mediated diseases, prevention of oxidative reactions in foods, protection against DNA damage and carcinogenesis, and possible substances with wide range of pharmacological activities such as anti-inflammatory, anti-bacterial, and anti-fungal properties. [10]. Natural products are defined as natural sources-derived substances having biological activities. Natural products have long been implemented as alternative health care treatment and in discovery of modern drugs [11]. A major focus of natural product chemistry has been toward drug design and discovery. Medicinal plants refer to the

class of plants applied for therapy or to possess pharmacological actions for human and animal [12]. Further investigation on isolation and characterization of bioactive compounds derived from natural extracts is in progress.

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