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Antioxidant Activity of Carica Papaya Leaf Extract (Rajasthan Variety).

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Abstract: ***Phytochemicals have the potential to stimulate the immune system, prevent toxic substances in the diet from becoming carcinogenic, reduce inflammation, prevent DNA damage and aid DNA repair, reduce oxidative damage to cells, slow the growth rate of cancer cells, trigger damaged cells to self-destruct (apoptosis) before they can reproduce, help regulate intracellular signaling of hormones and gene expression, and activate insulin receptors. One of the major functions of phytochemicals is their role as antioxidants. Antioxidant activity plays a major role in protecting our cells from oxidative damage which will lead to cancer. An attempt has been made to evolve the antioxidant activity of phytochemicals present in carica papaya leaf extract (Wild Species). Different concentrations of Carica papaya leaf extract (Wild Species) were prepared and allowed to stand for 30 minutes at room temperature. The antioxidant activity was studied at 517 nm by DPPH free radical scavenging activity.***

Keywords: Phytochemicals, Biologically active compounds, Antioxidant activity (AOA), Carica papaya leaf extract (CPLE), DPPH free radical scavenging activity (DPPH FSA).

I. INTRODUCTION

Commonly and erroneously referred to as a "tree", the plant is properly a large herb growing at the rate of 6 to 10 ft (1.8-3 m) the first year and reaching 20 or even 30 ft (6-9 m) in height, with a hollow green or deep-purple stem becoming 12 to 16 in (30-40 cm) or more thick at the base and roughened by leaf scars. Plants are used as medicine since time immemorial. It has been estimated that only 10- 15 percentage of the 7,50,000 plants have been surveyed for biologically active compounds. Approximately one- third of the top- selling drugs in the world are natural products. Among those all papaya which is botanically called as carica papaya has been administrated from ancient times since it is a rich source of vitamins, minerals and phytochemicals. Papaya belongs to the tropics of Americas. Papaya tree can grow upto 5 to 10 m. Its leaves are in the diameter of 50-70 cm with seven lobes. Its fruit is 10-30 cm in diameter **[1]**. Papaya consists of vitamin A, E and vitamin C along with minerals Magnesium, Potassium, Iron and Calcium. These vitamins reduce the severity of diseases like Asthma, Osteoarthritis and rheumatoid. Other than minerals and vitamins papaya is a rich source of phytochemicals such as terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins, steroids etc. These phytochemicals play a vital role against various diseases.

Phyto constituents are the natural bioactive compounds found in plants. These phyto constituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions (Koche *et al.*, 2010).[2] These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others (Nonita *et al.*, 2010).[3] Antioxidants are the substances which inhibit oxidation, which have the ability to remove the potentially damaging oxidizing agents in a living organism. Many phytochemicals present in the plants are able to reduce or prevent the oxidative damage to the human cells which can cause even cancer in humans. It is highly vital to know about the antioxidant activities of each plant and the phyto compounds responsible for that. Free radicals are atoms or groups of atoms that have at least one unpaired electron, which makes them highly reactive. Free radicals promote beneficial oxidation that produces energy and kills bacterial invaders. In excess, however, they produce harmful oxidation that can damage cell membranes and cell contents. It is known that people who eat adequate amounts of fruits and vegetables high in antioxidants have a lower incidence of cardiovascular disease, certain cancers, and cataracts. Fruits and vegetables are rich in antioxidants, but it is not known which dietary factors are responsible for the beneficial effects. Each plant contains hundreds of phytochemicals (plant chemicals) whose presence is dictated by hereditary factors. Only well-designed long-term research can determine whether any of these chemicals, taken in a pill, would be useful for preventing any disease.

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There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources (Abdalla and Roozen, 1999).[4] There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants (Sanchez-Moreno, 2002; Schwarz, et al., 2001).[5] One such method that is currently popular is based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH).

DPPH - free radical and reduced form:

The molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH: 1) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals.

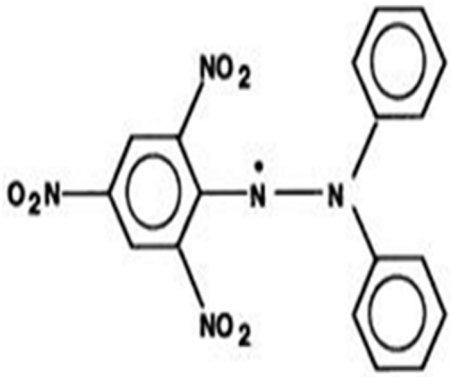

Fig.1: Diphenylpicrylhydrazyl (free radical)

The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centred at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (2) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present). Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is

$$Z^{\bullet} + AH = ZH + A^{\bullet} \text{ ----------[1]}$$

where ZH is the reduced form and A• is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant. The reaction [1] is therefore intended to provide the link with the reactions taking place in an oxidising system, such as the autoxidation of a lipid or other unsaturated substance; the DPPH molecule Z• is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by the substance AH.[6]

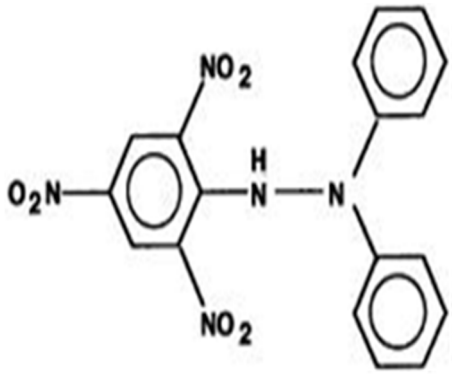

Fig 2: Diphenylpicrylhydrazyl (non radical)

A. Objective of research

The main objective of my research is to prove the antioxidant activity of Carica papaya leaf extract (Wild Species) and to examine whether the antioxidant activity increases as the concentration increases. By proving this I want to identify whether the extract is safe for invitro and invivo use?

B. Justification of research:

Free radical is any atom with atleast one unpaired electron in the outermost shell and is capable of independent existence. Free radicals and other reactive species produced during aerobic metabolism in the body can cause oxidative damage of amino acids, lipids, proteins and DNA[7]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others. Interestingly the body possesses defense mechanisms against free radical induced oxidative stress which involve preventative mechanisms, repair mechanisms, physical effects and antioxidant defenses. Mainly the antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the aging process [8]. Antioxidants may protect the body against ROS toxicity either by preventing the formation of ROS, by bringing interruption in ROS attack, by scavenging the reactive metabolites or by converting them to less reactive molecules. The antioxidant capacity gives information about the duration while the activity describes the starting dynamics of antioxidant action. Therefore the uses of antioxidants, both natural and synthetic are gaining wide importance in prevention of diseases [9].

II. MATERIALS AND METHODS

Hybrid Carica papaya.mettur variety-1 leaves were collected from villages in the month of November. The plant material was identified and authenticated by The Rapinat Herbarium and Center for molecular Systematics in St. Joseph College (*Autonomous*), Tiruchirappalli. The plant leaves were shade dried at ambient temperature 31^0C and the dried materials were crushed into fine powder using an electric blender.

A. Mode of collection

About 60 gm of dry sample powder was weighed and mixed with 500 ml of each solvent (Ethanol) separately and kept overnight in shaker for about 48 hours. The extract was collected after filtration using Whatman No.1 filter paper and was stored. The crude plant extract was extracted by using Soxhlet apparatus and was concentrated to dryness in vaccum evaporator below 50^0C and stored until needed for the bioassays at -4^0C.

B. In Vitro Antioxidant Activity

1) DPPH radical-scavenging activity: DPPH radical-scavenging activity was determined by the method of Shimada *et al.*, (1992).

2) Reagents:

a) DPPH : 25 µg/ml in methanol

b) Methanol

3) Procedure: Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = 100 - \left(\frac{A_C - A_S}{A_C} \times 100 \right)$$

Where $A_{C\,=}$ control is the absorbance and $A_{S\,=}$ sample is the absorbance of reaction mixture (in the presence of sample). (10)

C. Statistical analysis

One parameter that has been introduced recently for the interpretation of the results from the DPPH method, is the "efficient concentration" or EC50 value (otherwise called the IC50 value). This is defined as the concentration of substrate that causes 50% loss of the DPPH activity (colour). This parameter was apparently introduced by Brand-Williams and his colleagues (BrandWilliams et al., 1995; Bondet et al., 1997), and has been used subsequently by several groups of workers for presenting their results (Kim et al., 2002; Lebeau et al., 2000; Leit a˜ o et al., 2002; Lu and Foo, 2000; Sa ′ nchez-Moreno et al., 1998; Sa ′ nchez-Moreno et al., 1999). As a term, it was presumably introduced on analogy with "biological" parameters such as IC_{50}.[6]

III. RESULTS

Table 1 DPPH radical scavenging activity of plant extract

Concentration (µg/ ml)	Plant extract	Standard (Ascorbic acid)
20	26.93 ± 1.88	22.6 ± 2.04
40	34.62± 2.42	53.26 ± 4.90
60	53.85 ± 3.76	78.98 ± 7.11
80	73.08 ± 5.11	89.34 ± 7.94
IC_{50}	53.68	40.24

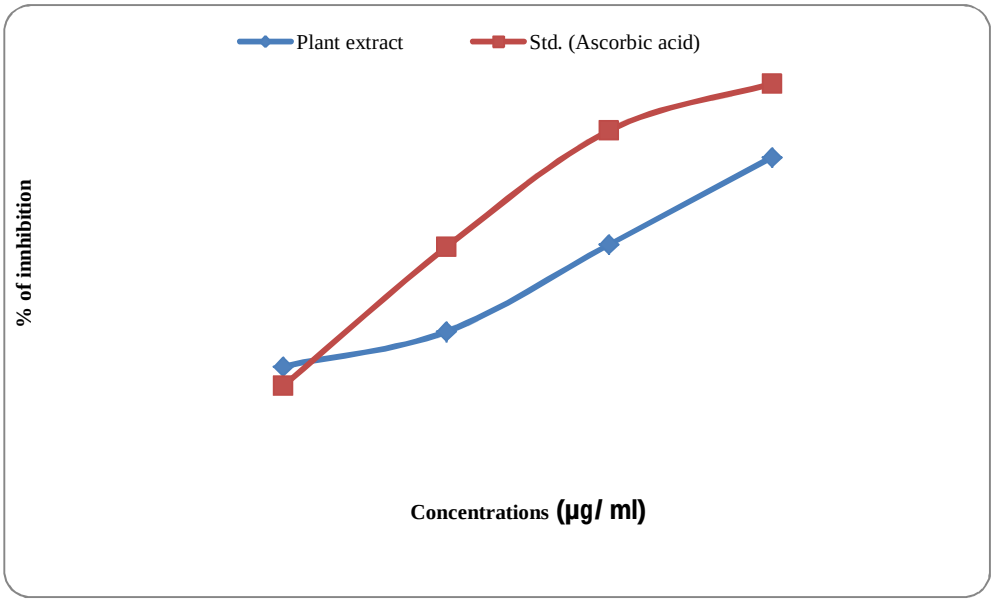

Fig 3 DPPH radical scavenging activity of plant extract

The antioxidant activities were determined using DPPH as free radical. The antioxidant activities were read at various concentrations using ascorbic acid as standard. Tests were carried out in triplicate for 3 separate experiments. The scavenging activity of sample was expressed as 50% effective concentration (EC_{50}), which represented the concentration of sample having 50% of radical scavenging effect. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined by a linear regression method using Ms- Windows based graph pad instat (version 3) software. Results were expressed as graphically / Mean± standard deviation. The various concentrations are taken along the X-axis while the percentage inhibition is taken along the Y- axis. From the above graph it is clear that the carica papaya leaf extract is found to have antioxidant activity and it is having ability to prevent the cell damage. The antioxidant activity may also protect the human body from the development of the deadly disease known as cancer.

IV. CONCLUSION

The present study was carried out to analyze the antioxidant activity of the plant Carica papaya leaf extract (Wild Species). The scavenging activity of the plant extract through the annihilation of the DPPH radicals was investigated from the above study I can clearly say that Carica papaya leaf extract (Wild Species) is found to have the antioxidant activity. The antioxidant activity of the Carica papaya leaf extract (Wild Species) increases as the concentration increases. Higher concentration of carica leaf extract shows higher antioxidant activity. The antioxidant activity may also prevent the development of deadly disease known as cancer. Hence the antioxidant activity of carica is proven.

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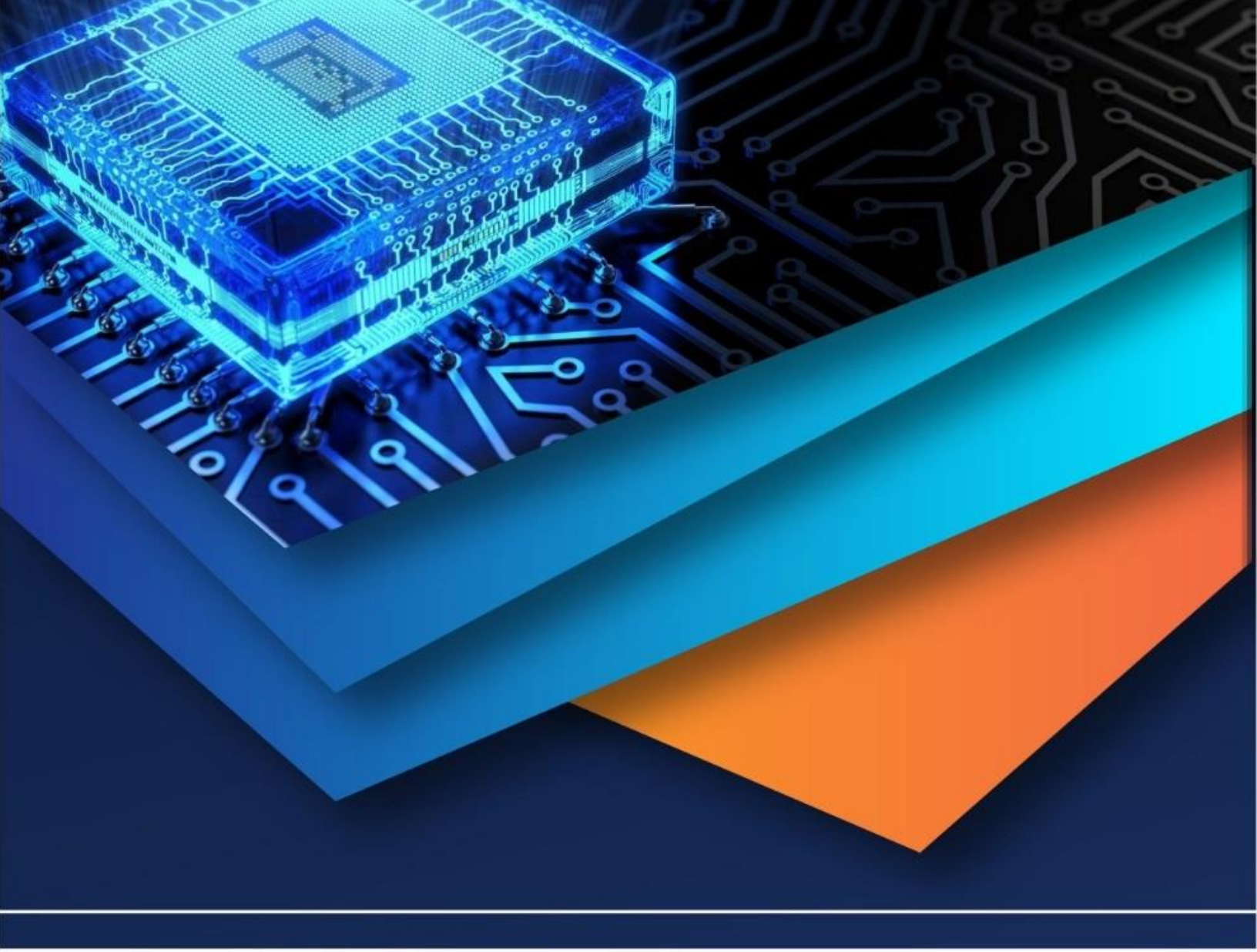

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