# COMPARATIVE ANTIOXIDANT STUDY OF ALLIUM SATIVUM LEAVES CROPPED IN PAKISTAN VERSUS WELL KNOWN STANDARD ANTIOXIDANT AGENT

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## ABSTRACT

**Objectives:** To reveal harmless noble natural antioxidant products to alleviate the extreme oxidant stress like ageing, cancer, heart and kidney maladies. **Place of study:** In the division of drug store, UOL, Food and Biotechnology Lab. of (PCSIR), Lahore. **Concentrate Rational:** The arrangement of present investigation was to gauge and think about the cell reinforcement impact of various concentration of Allium sativum leaves extricate with that of standard antioxidant agent Vitamin 'C' on 4% DPPH methanol solution. **Method:** 48 sterilized test tubes were divided into seven groups. Out of seven groups, two groups were kept as control groups and remaining five were used for experimental study of different concentration of Allium sativum leaves extract in comparison to that of vitamin C on 4% DPPH of methanol solution. **Result:** 54  $\mu$ g/mL of the Allium sativum leaves extricate concentration demonstrated antioxidant IC<sub>50</sub> (50% inhibitory concentration) in the present investigation. **Conclusion:** the antioxidant IC<sub>50</sub> of Allium sativum leaves indicates that its intake may help the living organism to battle against various antioxidant stress conditions and to prevent cancer/ to forestall abnormal growth.

**Key words:** Allium sativum, DPPH (2, 2-diphenyl-1-picrylhydrazyl), antioxidant agents, Vitamin C as antioxidant, etiology of cancer, mechanism of aging, antioxidant and heart disease, antioxidant and kidney disease, leaves of Allium Sativum (A Sativum).,  $IC_{50}$  (50% inhibitory concentration).

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## INTRODUCTION

In living organisms, the oxidation of organic molecules occur in normal biochemical metabolism that lead the creation of free radicals and receptive oxygen species (ROS) which are very harmful in many ways. e. g. they may start a chain of reactions that can damage organelles (like RNA, DNA, Mitochondria), of the cells . They catalyze a series of reactions that will initiate improper functioning of cell, and may lead to apoptosis or early cell death.<sup>1</sup> Excessive and fast production (oxidative stress) of free radicals and reactive oxygen species (ROS) occur during body biochemical reactions, when livings take excessive food, polluted air/light, bad dietary habits like smoking, drinking and work stress.<sup>2</sup> Oxidative stress may initiate different complications including body ageing, cancer, arthritis, cataract, diabetes heart, kidney and Alzheimer diseases.

Particles that subdue the procedure of oxidation in the body are called cell reinforcements (antioxidants) and have multiple beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic and antitumor movement<sup>2</sup> Thiobarbituric acid (TBA) method, DPPH, Chelating of minerals, Reducing power, Oxygen radical absorbance capacity (ORAC) are various procedures to evaluate antioxidant activity of plants.<sup>3</sup> To check phenolic compounds in plant extract, DPPH model is a more frequent method to determine the antioxidant activity.<sup>4</sup> Moreover, DPPH method is the most simple, easy and low cost procedure to determine the antioxidant activity of a sample<sup>3</sup>.

Therefore in the present investigation, the antioxidant capability of Allium sativum (A. Sativum) leaves were measured by using DPPH model in the Food and Biotechnology Lab. of PCSIR.

## **MATERIAL AND METHODS**

**Preapproval** was obtained from institutional review board (IRB) of UOL and PCSIR.

Forty eight sterilized test tubes (Image -1) were divided into seven groups named as 'A', 'B', 'C', 'D', 'E', 'F' and 'G'. Group 'A' and 'B' were kept as placebo control groups . while group 'C', 'D', 'E', 'F' and 'G' were kept as experimental groups<sup>5</sup> . Each control group contained four test tubes while there were eight tubes in each experimental group. The test tubes in group 'A' kept were labeled as 1 to 4. Each test tube of this group contained 3ml methanol and kept as placebo control of solvent.<sup>6</sup> The test tubes kept in group 'B' were labeled as 5, to 8. Each test tube in this group contained 3ml of 4% DPPH methanol solution and kept as placebo control of DPPH & solvent.<sup>7</sup>

The test tubes of group 'C' labeled as 9 to 12 were having  $18\mu$ g/ ml of aqueous garlic leaves extract in 3ml 4% DPPH methanol solution while the test tubes of group C labeled as 13 - 16 were having  $18\mu$ g / ml of vitamin C in 3ml 4% DPPH methanol solution.<sup>8</sup> The test tubes of group 'D' labeled as 17 to 20 were having  $36\mu$ g/ ml of aqueous garlic leaves extract in 3ml 4% DPPH methanol solution while its test tubes labeled as 21 to 24 were having  $36\mu$ g/ ml of vitamin C in 3ml 4% DPPH methanol solution.<sup>9</sup>

The test tubes of group 'E' labeled as 25 to 28 were

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having  $54\mu g/ml$  of aqueous garlic leaves extract in 3ml 4% DPPH methanol solution while its test tubes labeled as 29- to 32 were having  $54\mu g/ml$  of vitamin C in 3ml 4% DPPH methanol solution.<sup>10</sup> The test tubes of group 'F' labeled as 33 to 36 were having  $72\mu g/ml$  of aqueous garlic leaves extract in 3ml 4% DPPH methanol solution while its test tubes labeled as 37 to 40 were having  $72\mu g/ml$  of vitamin C in 3ml 4% DPPH methanol solution.<sup>11</sup>

The test tubes of group 'G' labeled as 40 to 44 were having  $90\mu g/ml$  of aqueous garlic leaves extract in 3ml 4% DPPH methanol solution while its test tubes labeled as 45,46,47 and 48 were having  $90\mu g/ml$  of vitamin C in 3ml 4% DPPH methanol solution.<sup>12</sup>

All the test containers of different groups were sealed, vortexed and kept for 30 minute at room temperature.<sup>13</sup> The absorbance of all samples of the present study was assayed at 517 nm in 1-cm quartz cell of an UIKON spectrophotometer following the William's procedure<sup>10</sup>.

## RESULT

The comparative  $IC_{50}$  (50% inhibitory concentration) of the aqueous garlic leaves extract with ascorbic acid resulted in the present study was 54 µg/ml.<sup>14</sup>

Bar Chart (fig.1) is drawn to determine comparative Values of concentration for *Allium sativum* leaves and ascorbic acid of present study in relation to the absorbance.







Figure 02: was made to determine  $IC_{\mathfrak{so}}$  of Allium sativum leaves in comparison with that of vitamin 'C'

# **S**tatistical analysis

The data of antioxidant activity of garlic leaves were analyzed by One way ANOVA with multiple comparison which showed significant higher (P < 0.05).<sup>15</sup>

## DISCUSSION

A perpetual herb Allium sativum (garlic), normally has a place in Alliaceae (the onion family)<sup>15</sup>. For cooking purpose, it is used all over the world in various ways due to its typical flavor, aroma, taste and nutrition in baked foods, meat, fish, pickles, soups, sauces. It has been also being in use for health restorative purposes since long ago due to its certain active ingredients.<sup>16</sup>

One reason of Its selection for present study was its cheapest cost, easy accessibility and cultivation all over the world.<sup>17</sup> Though many synthetic antioxidants are easily available in the market for research purpose but were not be selected for the present study because of their disadvantages and their conceivable harmful properties for human and creature well being.<sup>18</sup>

The reinforcement (antioxidant) value of aqueous garlic leaves extract was carried out by utilizing DPPH technique with Vitamin C taken as standard and found higher radical chasing activity of garlic extract. The mechanism that how do DPPH facilitate In determination of capacity of reinforcement of different concentrates of the sample is that the release hydrogen atoms or electrons by the sample substance are being absorbed by DPPH. It change DPPH radical into its diminished shape DPPH.H.<sup>19</sup> When this reduction reaches 50% (called  $IC_{50}$ ), it decrease the steady purple colour of radical (DPPH) into the yellow-shaded (diminished DPPH.H). in the present investigation, the amount of Ascorbic acid used as standard whose IC<sub>50</sub> value was 22.78  $\underline{ug}$  mL<sup>-1</sup> while the leaves of the variety of A. Sativum commonly cropped in Pakistan was found 54  $\mu$ g/ml.<sup>20</sup> This value is a little bit different and better than that of China and Indian's A. Sativum crops because IC<sub>50</sub> of china and Indian A. Sativum leaves crops are 60  $\mu$ g/mL and 65  $\mu$ g/mL respectively i.e more than that of Pakistan's A. Sativum.<sup>21</sup> in the present investigation, IC50 value was determined not only by the color method but was also measured by absorbance at 517 nm in 1-cm guartz cell of an UIKON spectrophotometer by the William's procedure.<sup>13</sup> It is informative also in spite of higher reinforcement value of A. Sativum leaves than of garlic bulbs, these leaves are commonly be wasted by our community.<sup>22</sup>

Dynamic elements of A. Sativum incorporate 17 amino acids and 33 sulfur compounds. These sulphur compounds prove health restorative effects and responsible for its pungent odor characteristic aroma.<sup>23</sup> The most rich assortment of sulfur mixes in garlic is alliin (S-allylcysteine sulfoxide), progress toward becoming allicin in newly pounded garlic homogenates that has that has significant impacts by improving the serum levels of two cell reinforcement chemicals, catalase and glutathione peroxidase.<sup>24</sup>

## CONCLUSION

The present investigation uncovered the reinforcement (antioxidant) capability of garlic leaves. The IC<sub>50</sub> of garlic leaves extract in present study found was  $54 \mu g/ml$ . The antioxidant result indicate the potential of these leaves equip the body to combat against various stress conditions including anti-microbial effect.

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