

## IN VITRO ANTIOXIDANT ACTIVITY AND SCAVENGING EFFECTS OF SOME NEW 1,3,5-TRIPHENYL- 2-PYRAZOLINES AND 3-(2''-HYDROXY NAPHTHALEN-1''-YL)-1,5-DIPHENYL-2-PYRAZOLINES

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تم تشييد وتقييم طائفة جديدة من مشتقات بيرازولين الواعدة من حيث فاعليتها المضادة للأكسدة. ولقد تم تقييم خمسة عشر مركباً جديداً منها باستخدام أنواع مختلفة من طرائق تحليله للأكسجين النشط مختلفة محتوية على أيون سوبرأوكسيد السالب، وأنيون هيدروكسيل، وشق DPPH، وتحليل كاسحات شق كاتيون ABTS، وطريقة تحليل بتثبيط فوق أكسدة الشحوم. وقد أظهرت المركبات تأثيرات واضحة في طرائق تحليل كسح الشقوق الحرة وتثبيط فوق أكسدة الشحوم. المركبان 10، 13 اللذان تم بهما إحلال مجموعة ميثوكسي الثلاثية على حلقة فنييل في الموقع 5 لمجموعات 2-بيرازولين، أظهرتا أعلى فاعلية أكثر من الدواء القياسي وهو حمض أسكوربيك. أما المركبات 1، 2، 3، 4، 11 والتي توجد بها مجموعات داي ميثيل أمينو في الموقع 4 من حلقة فنييل لمجموعات 2-بيرازولين فإنها أظهرت فاعلية جيدة. وقد كشفت هذه الدراسة أن المجموعة المطلقة للإلكترون في الموقع 4 حلقة فنييل ضرورية جداً لكل مجموعات 2-بيرازولين لكي تبدي فاعلية ملحوظة.

A new class of potential pyrazoline derivatives had been synthesized and evaluated for their anti oxidant activities. The obtained five new 1,3,5-triphenyl-2-pyrazolines and another ten 3-(2''-hydroxynaphthalen-1''-yl)-1,5-diphenyl-2-pyrazolines were evaluated for antioxidative activities by using different reactive oxygen species (ROS) assays containing superoxide anion, hydroxyl radical, ABTS cation radical scavenging assays and inhibition of lipid peroxidation assay. The compounds exhibited more prominent effects in scavenging the free radical assays and also inhibited the lipid peroxidation. Compounds 10,13 possessing trimethoxy substitution on phenyl ring at position 5 of the 2-pyrazolines exhibited maximum activity, infact more than that of the standard drug ascorbic acid. Compounds 11, 3, 4, 2 and 1 having dimethyl amino groups at position 4 of the phenyl ring at position 5 of the 2-pyrazolines also showed good activity. The present study revealed that an electron releasing group at position 4 of the phenyl ring is very much essential for all these 2-pyrazolines to show significant activity.

**Key words :** Pyrazoline, superoxide, hydroxyl, antioxidant, lipid peroxidation.

### Introduction

The role of free radicals has been participated in the cause of several diseases such as liver cirrhosis,

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atherosclerosis, cancer, aging, arthritis, diabetes, etc (1) and the compounds that can scavenge free radicals have great potential in ameliorating these disease processes (2). The human body has inherent mechanisms to reduce free radical induced injury by endogenous enzymes such as superoxide dismutase, glutathione peroxidase, catalase, and other such as vitamin C (ascorbic acid), etc. Sometimes these protective mechanisms are found not to be sufficient

when compared to the damage occurred to the body. Hence, the search for exogenous antioxidants is continuing (3). The ROS include superoxide anions ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radicals ( $OH$ ). Lipid peroxidation, which involves a series of free radical mediated chain reactions processes, is also associated with several types of biological damage. Therefore much attention has been focused on the use of synthetic antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals.

Considerable interest has been focused on the pyrazoline structure, which has been known to possess a broad spectrum of biological activities such as tranquillizing, muscle relaxant, psychoanaesthetic, anticonvulsant, antihypertensive and antidepressant activities (4-9). The discovery of this class of compounds provides an outstanding case history of modern drug development and also points out the unpredictability of biological activity from structural modification of a prototype leading molecule. As part of our continuing efforts in this area, a series of some new 1-phenyl-3-(2'' and/or 4''-substituted phenyl)-5-(3'-and/or 4'-substituted phenyl)-2-pyrazolines and 1-(phenyl)-5-(2'-and/or 3'-and /or 4'-substituted phenyl)-3-(2''-hydroxy-naphthalen-1''-yl)-2-pyrazolines had been synthesized and evaluated for their antidepressant activities (10). The present paper was aimed to investigate the free radical scavenging and antioxidant activities of 1, 3, 5-triphenyl-2-pyrazolines and 3-(2''-hydroxynaphthalen-1''-yl)-1,5-diphenyl-2-pyrazolines.

### Experimental

#### Chemicals:

2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was purchased from Sigma-Aldrich (Hyderabad, India). Nitroblue tetrazolium (NBT) was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, 2-deoxy-D-ribose was purchased from Sigma Chemical Company (USA). Riboflavin, L-ascorbic acid were purchased from Loba chemicals, Mumbai. All other chemicals and reagents used were of analytical reagent quality.

#### Chemistry:

The 1, 3, 5-triphenyl-2-pyrazoline (**1-5**) and 3-(2''-hydroxynaphthalen-1''-yl)-1, 5-diphenyl-2-pyrazoline (**6-10**) derivatives used in the present study were previously synthesized in our laboratory. The

structures and biological data of the compounds were given in Table 1. Their chemical names are 5-(4'-dimethylaminophenyl)-1,3-diphenyl-2-pyrazoline (**1**), 1-phenyl-3-(4''-methylphenyl)-5-(4'-dimethylaminophenyl)-2-pyrazoline (**2**), 1-phenyl-3-(2''-hydroxyphenyl)-5-(4'-dimethylaminophenyl)-2-pyrazoline (**3**), 1-phenyl-3-(4''-bromophenyl)-5-(4'-dimethylaminophenyl)-2-pyrazoline (**4**), 1-phenyl-3-(4''-hydroxyphenyl)-5-(3'-bromophenyl)-2-pyrazoline (**5**), 1-phenyl-3-(2''-hydroxynaphthalen-1''-yl)-5-phenyl-2-pyrazoline (**6**), 1-phenyl-3-(2''-hydroxynaphthalen-1''-yl)-5-(4'-methoxyphenyl)-2-pyrazoline (**7**), 1-phenyl-3-(2''-hydroxynaphthalen-1''-yl)-5-(4'-chlorophenyl)-2-pyrazoline (**8**), 1-phenyl-3-(2''-hydroxynaphthalen-1''-yl)-5-(4'-bromophenyl)-2-pyrazoline (**9**), 1-phenyl-3-(2''-hydroxynaphthalen-1''-yl)-5-(3',4',5'-trimethoxyphenyl)-2-pyrazoline (**10**), 5-(4'-dimethylaminophenyl)-3-(2''-hydroxynaphthalen-1''-yl)-1-phenyl-2-pyrazoline (**11**), 5-(2'-chlorophenyl)-3-(2''-hydroxynaphthalen-1''-yl)-1-phenyl-2-pyrazoline (**12**), 5-(2', 4', 5'-trimethoxyphenyl)-3-(2''-hydroxynaphthalen-1''-yl)-1-phenyl-2-pyrazoline (**13**), 5-(4'-nitrophenyl)-3-(2''-hydroxynaphthalen-1''-yl)-1-phenyl-2-pyrazoline (**14**), 5-(4'-methylphenyl)-3-(2''-hydroxynaphthalen-1''-yl)-1-phenyl-2-pyrazoline (**15**).

#### General procedure for the preparation of 2-pyrazolines (**1-15**):

To the solution of the appropriate chalcones (1 mmol) and phenyl hydrazine HCl (500mg) in ethanol (20 mL), pyridine (0.3 mL) was added as a catalyst. The mixture was refluxed for 4-6 h and the solvent was evaporated completely. The reaction mixture was poured into ice cold water and the solid mass that separated out was filtered, dried and purified by using column chromatography.

#### 5-(4'-dimethylaminophenyl)-3-(2''-hydroxynaphthalen-1''-yl)-1-phenyl-2-pyrazoline (**11**):

Yield 78%; mp 260-262 °C; IR (KBr) 3115 (OH), 1642 (C=N), 1350 (C-N);  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  9.65 (1H, s, C-2''-OH), 9.45 (1H, d,  $J=16$  Hz, C-8''-H), 6.90-7.80 (14H, m, Ar-H), 5.20 (1H, dd,  $H_X$ ), 4.10 (1H, dd,  $H_B$ ), 3.29 (1H, dd,  $H_A$ ), 2.89 (6H, s, -N(CH<sub>3</sub>)<sub>2</sub>), ( $J_{AB}=17.12$ ,  $J_{AX}=7.30$ ,  $J_{BX}=10.14$  Hz) MS (m/z): 407 ( $M^+$ ); MS (m/z): 407 ( $M^+$ ); Anal. Calcd for C<sub>27</sub>H<sub>25</sub>N<sub>3</sub>O: C, 79.56; H, 6.18; N, 10.31. Found: C, 79.38; H, 6.08; N, 10.22.

**5-(2'-chlorophenyl)-3-(2''-hydroxynaphthalene-1''-yl)-1-phenyl-2-pyrazoline (12):**

Yield 83%; mp 244-246 °C; IR (KBr) 3050 (OH), 1645 (C=N), 1350 (C-N), 855 (C-Cl); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.50 (1H, s, C-2'' -OH), 9.70 (1H, d, J=8 Hz, C-8''-H), 6.70-7.95 (14H, m, Ar-H), 5.15 (1H, dd, H<sub>X</sub>), 3.30 (1H, dd, H<sub>B</sub>), 3.00 (1H, dd, H<sub>A</sub>), (J<sub>AB</sub>=17.10, J<sub>AX</sub>=7.20, J<sub>BX</sub>=10.12 Hz) MS (m/z): 398.5 (M<sup>+</sup>); Anal. Calcd for C<sub>25</sub>H<sub>19</sub>N<sub>2</sub>OCl: C, 75.27; H, 4.80; N, 7.02. Found: C, 75.58; H, 4.38; N, 7.36.

**5-(2',4',5'-trimethoxyphenyl)-3-(2''-hydroxynaphthalene-1''-yl)-1-phenyl-2-pyrazoline (13):**

Yield 68%; mp 294-296 °C; IR (KBr) 3120 (OH), 2900 (C-H aliphatic stretch of -OCH<sub>3</sub>), 1640 (C=N), 1360 (C-N), 1180 (OCH<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.50 (1H, s, C-2'' -OH), 9.72 (1H, d, J=8 Hz, C-8''-H), 6.90-7.95 (12H, m, Ar-H), 5.30 (1H, dd, H<sub>X</sub>), 3.76 (3H, s, -OCH<sub>3</sub>), 3.69 (6H, s, 2X-OCH<sub>3</sub>), 3.30 (1H, dd, H<sub>B</sub>), 2.90 (1H, dd, H<sub>A</sub>), (J<sub>AB</sub>=17.38, J<sub>AX</sub>=7.48, J<sub>BX</sub>=9.62 Hz) MS (m/z): 454 (M<sup>+</sup>); Anal. Calcd for C<sub>28</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>: C, 73.99; H, 5.76; N, 6.16. Found: C, 73.72; H, 5.29; N, 6.56.

**5-(4'-nitrophenyl)-3-(2''-hydroxynaphthalene-1''-yl)-1-phenyl-2-pyrazoline (14)**

Yield 86%; mp 266-268°C; IR (KBr) 3050 (OH), 1645 (C=N), 1520 (Assymmetric stretch of ArNO<sub>2</sub>), 1340 (Symmetric stretch of ArNO<sub>2</sub>), 1350 (C-N); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 13.50 (1H, s, C-2'' -OH), 9.50 (1H, d, J=8 Hz, C-8''-H), 8.10-8.20 (2H, dd, C-3'-H, C-5'-H), 6.80-7.80 (14H, m, Ar-H), 5.30 (1H, dd, H<sub>X</sub>), 3.40 (1H, dd, H<sub>B</sub>), 3.10 (1H, dd, H<sub>A</sub>), (J<sub>AB</sub>=16.88, J<sub>AX</sub>=7.89, J<sub>BX</sub>=10.25 Hz); MS (m/z): 409 (M<sup>+</sup>); Anal. Calcd for C<sub>25</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>: C, 73.33; H, 4.67; N, 10.26. Found: C, 73.72; H, 5.09; N, 10.49.

**5-(4'-methylphenyl)-3-(2''-hydroxynaphthalene-1''-yl)-1-phenyl-2-pyrazoline (15)**

Yield 76%; mp 202-204°C; IR (KBr) 3100 (OH), 1642 (C=N), 1355 (C-N); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 12.35 (1H, s, C-2'' -OH), 9.60 (1H, d, J=8 Hz, C-8''-H), 6.80-8.00 (14H, m, Ar-H), 5.25 (1H, dd, H<sub>X</sub>), 4.31 (1H, dd, H<sub>B</sub>), 3.78 (1H, dd, H<sub>A</sub>), 2.40 (3H, s, -CH<sub>3</sub>), (J<sub>AB</sub>=16.80, J<sub>AX</sub>=7.85, J<sub>BX</sub>=10.20 Hz); MS (m/z): 378 (M<sup>+</sup>); Anal. Calcd for C<sub>26</sub>H<sub>22</sub>N<sub>2</sub>O: C, 82.51; H, 5.85; N, 7.40. Found: C, 82.38; H, 5.66; N, 7.53.

**Determination of Superoxide Scavenging Activity:**

Riboflavin Photoreduction Method: Superoxide Scavenging activity of the compounds was determined by McCord and Fridovich method (11), which depends on light induced superoxide generation by riboflavin and the corresponding reduction of Nitro blue tetrazolium (NBT). The assay mixture contained different quantities of the compounds and ethylene diamine tetraacetic acid (6μM containing 0.061μM NaCN), NBT (50 μM), riboflavin (2 μM) and phosphate buffer (58 mM, pH 7.8) to give a total volume of 3 ml. The tubes were uniformly illuminated with an incandescent light (40 Watts) for 15 minutes and thereafter the optical density was measured at 560 nm. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes. The inhibitory effects of samples on the generation of superoxide anion were estimated by the equation:

$$\text{Inhibitory ratio} = \frac{(A_0 - A_1) \times 100}{A_0}$$

Where, A<sub>0</sub> is the absorbance with no addition of sample; A<sub>1</sub> is the absorbance with addition of sample.

**Determination of Hydroxyl radical scavenging activity:**

Deoxyribose degradation Method: Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the compounds for the hydroxyl radical generated from the Fe<sup>+3</sup>- ascorbate- EDTA- H<sub>2</sub>O<sub>2</sub> system (12). The hydroxyl radical attacks deoxyribose and eventually results in thiobarbituric acid reacting substances (TBARS) formation. The reaction mixture containing deoxyribose (2.8 mM), ferric chloride (0.1mM), EDTA (0.1 mM), H<sub>2</sub>O<sub>2</sub> (1 mM), ascorbate (0.1mM), phosphate buffer (20 mM, pH 7.4) and various quantities of the compounds in a final volume of 1 ml was incubated for 1 hour at 37°C. Deoxyribose degradation was measured as TBARS by the method of Ohkawa *et al.*, (13). The percentage of inhibition was calculated from the control where no test compound was added.

**Determination of Lipid Peroxidation Inhibition Activity:**

Induction by Fe<sup>2+</sup>/Ascorbate System: Inhibition of lipid Peroxidation was determined by the thio-

barbituric acid method (13). Different quantities of the compounds were incubated at 37°C with 25% (W/V) rat liver homogenate (0.1 ml) containing Tris-Buffer (40 mM, pH 7.0), KCl (30 mM), ascorbic acid (0.06 mM), and ammonium ferrous sulphate (0.16 mM) in a total volume of 0.5 ml for 1 hour. At the end of the incubation period, 0.4 ml of the reaction mixture was treated with 0.2 ml of sodium dodecyl sulphate (8.1%), 1.5 ml thiobarbituric acid (0.8%), and 1.5 ml of acetic acid (20%, pH 3.5). The total volume was then made up to 4 ml by adding distilled water and kept in an oil bath at 95°C for 1 hour. After the mixture had been cooled, 1 ml of distilled water and 5 ml of butanol-pyridine mixture (15:1 v/v) was added. Following vigorous shaking, the tubes were centrifuged and the absorbance of the upper layer containing the chromophore was read at 532 nm. The percentage inhibition of lipid Peroxidation was determined by comparing the absorbance values of the control and experimental tubes.

#### *ABTS<sup>+</sup> radical cation scavenging activity:*

The ABTS<sup>+</sup> radical cation scavenging activity of test compounds, Ascorbic acid (as standard) was determined according to Re *et al.*, (14). Briefly, 5.0 ml of 7.0 mM ABTS was reacted with 88.0 µl of 140 mM potassium persulfate overnight in the dark to yield the ABTS<sup>+</sup> radical cation. Prior to use in the assay, the ABTS<sup>+</sup> radical cation diluted with 50% ethanol for an initial absorbance of ≈0.700 (1:88 ratio) at 734 nm, with temperature control set at 30°C. Free radical scavenging activity was assessed by mixing 1.0 ml diluted ABTS<sup>+</sup> radical cation with 10 µl of test antioxidant and monitoring the change in absorbance at 0, 0.5, 1, min and again at 5 min intervals until a steady state was achieved. The antioxidant capacity of test compounds was expressed as EC<sub>50</sub>, the concentration necessary for 50% reduction of ABTS<sup>+</sup>.

## Results

#### *Superoxide scavenging activity:*

Compounds 1-15 were found to be scavenging the superoxides generated by photoreduction of riboflavin. Among them, compound 13, 10, 11, 3, 4, 2 & 1 produced a dose dependent inhibition of superoxide radicals at quantities of 1.5, 3, 4.5, 6, 7.5 & 10 µM. The remaining compounds exhibited less activity when compared to the above compounds at

similar concentrations. The mean values of inhibition for each compound at each concentration level are presented in Table 2. The quantities needed for 50% inhibition of superoxide radical activity by the compounds 1-15 were found to be 9.59, 9.13, 7.90, 8.00, 11.05, 11.83, 11.05, 12.34, 12.64, 5.53, 8.89, 10.63, 7.26, 10.82 and 9.39 µM respectively. Ascorbic acid, the known antioxidant employed in this study for comparison produced similar effect at a concentration of 9.89 µM. This clearly revealed that most of the compounds used in the study possess significant superoxide scavenging activity, in some cases even superior to ascorbic acid. Compound 10 appears to be the best among the tested.

#### *Hydroxyl scavenging activity:*

Degradation of deoxyribose mediated by hydroxyl radicals generated by Fe<sup>3+</sup>/ascorbate / EDTA/ H<sub>2</sub>O<sub>2</sub> system was found to be inhibited by the compounds tested. Table 3 displays the hydroxyl scavenging activity of the compounds when tested by the deoxyribose method and results are expressed as percentage inhibition of hydroxyl radical in relation to a control. The activity was measured over a broad range of concentrations in comparison to ascorbic acid. All the compounds produced a dose dependent hydroxyl scavenging activity and the quantities of these compounds 1-15 needed to produce 50% of this activity for were found to be 15.98, 12.99, 12.13, 12.67, 16.54, 18.60, 16.46, 18.65, 20.76, 8.91, 11.21, 13.87, 9.60, 14.43 and 13.25 µM respectively. In the case of ascorbic acid the quantities needed for 50% hydroxyl scavenging activity was 13.50 µM.

#### *Inhibition of lipid peroxidation:*

Lipid peroxides generated by the induction of Fe<sup>2+</sup>/Ascorbate on rat liver homogenate were found to be inhibited by the addition of compounds 1-15. Table 4 displays the percentage inhibition of lipid peroxidation of the compounds when tested by thiobarbituric acid method in relation to a control. Even though all compounds showed inhibition of lipid peroxidation, compounds 13, 10, 11, 3, 15, 12, 14, 4, 2 and 1 exhibited prominent and dose dependant inhibitions comparable to that of the standard drug ascorbic acid. The quantities of compounds 1-15 needed for 50% inhibition were found to be 48.36, 43.28, 28.55, 33.16, 49.34, 49.62, 48.46, 49.62, 52.62, 20.32, 28.28, 29.83, 20.82, 31.30 and 28.74 µM respectively. In case of

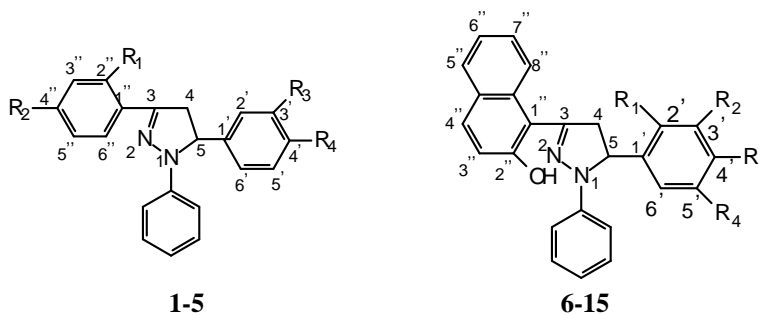
Ascorbic acid the quantity needed for 50% inhibition of lipid peroxidation activity was 30.10  $\mu\text{M}$ .

*ABTS<sup>+</sup> radical cation scavenging activity:*

The antioxidant ability to scavenge the ABTS<sup>+</sup> radical has been compared to the standard Ascorbic acid and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants. The percentage scavenging of ABTS<sup>+</sup> by the compounds at different concentrations were shown in Table.5.

Even though all compounds showed a dose dependent scavenging activity, compounds 13, 10, 11, 3, 4, 2 and 1 exhibited prominent and dose dependent inhibitions comparable to that of the standard drug ascorbic acid. The quantities of compounds 1-15 needed for 50% inhibition were found to be 8.39, 8.17, 7.36, 8.05, 10.66, 10.91, 8.51, 12.58, 13.26, 5.19, 7.39, 11.70, 6.14, 12.38 and 11.41  $\mu\text{M}$  respectively. In case of Ascorbic acid the quantity needed for 50% ABTS radical scavenging activity was 9.42  $\mu\text{M}$ .

**Table 1:** Structure and Biological data of the compounds 1-15.



|    | R <sub>1</sub>    | R <sub>2</sub>    | R <sub>3</sub>                    | R <sub>4</sub>                    | Superoxide<br>IC <sub>50</sub> Value | Hydroxyl<br>IC <sub>50</sub> Value | Lipid<br>peroxidation<br>IC <sub>50</sub> value | ABTS radical cation<br>IC <sub>50</sub> Value |
|----|-------------------|-------------------|-----------------------------------|-----------------------------------|--------------------------------------|------------------------------------|---|---|
| 1  | -H                | -H                | -H                                | -N(CH <sub>3</sub> ) <sub>2</sub> | 9.59± 1.1                            | 15.98± 1.9                         | 48.36± 3.1                                      | 8.39± 1.9                                     |
| 2  | -H                | -CH <sub>3</sub>  | -H                                | -N(CH <sub>3</sub> ) <sub>2</sub> | 9.13± 0.9                            | 12.99± 1.5                         | 43.28± 2.5                                      | 8.17± 1.6                                     |
| 3  | -OH               | -H                | -H                                | -N(CH <sub>3</sub> ) <sub>2</sub> | 7.90± 0.8                            | 12.13± 1.3                         | 28.55± 1.9                                      | 7.36± 1.2                                     |
| 4  | -H                | -Br               | -H                                | -N(CH <sub>3</sub> ) <sub>2</sub> | 8.00± 1.0                            | 12.67± 0.8                         | 33.16± 3.2                                      | 8.05± 1.3                                     |
| 5  | -H                | -OH               | -Br                               | -H                                | 11.05± 0.6                           | 16.54± 0.5                         | 49.34± 3.3                                      | 10.66± 1.7                                    |
| 6  | -H                | -H                | -H                                | -H                                | 11.83± 1.4                           | 18.60± 1.6                         | 49.62± 2.8                                      | 10.91± 2.4                                    |
| 7  | -H                | -H                | -OCH <sub>3</sub>                 | -H                                | 11.05± 1.6                           | 16.46± 1.7                         | 48.46± 2.6                                      | 8.51± 1.5                                     |
| 8  | -H                | -H                | -Cl                               | -H                                | 12.34± 1.5                           | 18.65± 0.9                         | 49.62± 3.2                                      | 12.58± 2.8                                    |
| 9  | -H                | -H                | -Br                               | -H                                | 12.64± 0.9                           | 20.76± 1.6                         | 52.62± 4.5                                      | 13.26± 2.9                                    |
| 10 | -H                | -OCH <sub>3</sub> | -OCH <sub>3</sub>                 | -OCH <sub>3</sub>                 | 5.53± 1.2                            | 8.91± 1.7                          | 20.32± 3.6                                      | 5.19± 0.6                                     |
| 11 | -H                | -H                | -N(CH <sub>3</sub> ) <sub>2</sub> | -H                                | 8.89± 1.3                            | 11.21± 2.7                         | 28.28± 2.9                                      | 7.39± 1.4                                     |
| 12 | -Cl               | -H                | -H                                | -H                                | 10.63± 0.7                           | 13.87± 2.9                         | 29.83± 3.5                                      | 11.70± 1.8                                    |
| 13 | -OCH <sub>3</sub> | -H                | -OCH <sub>3</sub>                 | -OCH <sub>3</sub>                 | 7.26± 1.4                            | 9.60± 3.1                          | 20.82± 2.8                                      | 6.14± 1.0                                     |
| 14 | -H                | -H                | -NO <sub>2</sub>                  | -H                                | 10.82± 1.5                           | 14.43± 1.5                         | 31.30± 2.2                                      | 12.38± 1.8                                    |
| 15 | -H                | -H                | -CH <sub>3</sub>                  | -H                                | 9.39± 1.3                            | 13.25± 1.8                         | 28.74± 2.6                                      | 11.41± 1.1                                    |

**Table 2.** Percentage inhibition of superoxide Radical using riboflavin photoreduction method.

| Compound      | Quantity ( $\mu\text{M}$ ) |                  |                  |                  |                  |                  |                  |
|---------------|----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|               | 1.5                        | 3                | 4.5              | 6                | 7.5              | 10               | 12.5             |
| 1             | 19.84 $\pm$ 1.81           | 25.43 $\pm$ 1.12 | 31.02 $\pm$ 2.23 | 36.60 $\pm$ 1.15 | 42.19 $\pm$ 0.57 | 51.51 $\pm$ 1.37 | 60.83 $\pm$ 0.66 |
| 2             | 13.05 $\pm$ 1.92           | 20.30 $\pm$ 2.18 | 27.56 $\pm$ 1.46 | 34.81 $\pm$ 1.56 | 42.07 $\pm$ 0.88 | 54.16 $\pm$ 0.99 | 66.25 $\pm$ 0.98 |
| 3             | 18.00 $\pm$ 2.12           | 25.49 $\pm$ 0.96 | 32.99 $\pm$ 2.32 | 40.48 $\pm$ 0.99 | 47.97 $\pm$ 1.34 | 60.46 $\pm$ 2.23 | 72.95 $\pm$ 1.26 |
| 4             | 13.72 $\pm$ 1.63           | 22.09 $\pm$ 1.19 | 30.45 $\pm$ 0.98 | 38.81 $\pm$ 0.83 | 47.18 $\pm$ 1.78 | 61.12 $\pm$ 0.68 | 75.05 $\pm$ 1.46 |
| 5             | 9.68 $\pm$ 1.75            | 16.01 $\pm$ 2.22 | 22.33 $\pm$ 0.86 | 28.66 $\pm$ 1.22 | 34.99 $\pm$ 2.01 | 45.53 $\pm$ 0.78 | 56.07 $\pm$ 1.42 |
| 6             | 9.50 $\pm$ 1.24            | 15.38 $\pm$ 0.88 | 21.26 $\pm$ 1.22 | 27.14 $\pm$ 1.48 | 33.02 $\pm$ 2.22 | 42.82 $\pm$ 1.01 | 52.61 $\pm$ 0.56 |
| 7             | 13.09 $\pm$ 1.53           | 18.88 $\pm$ 3.11 | 24.68 $\pm$ 0.89 | 30.47 $\pm$ 0.92 | 36.27 $\pm$ 0.99 | 45.92 $\pm$ 0.77 | 55.58 $\pm$ 0.78 |
| 8             | 6.66 $\pm$ 1.48            | 12.66 $\pm$ 2.22 | 18.65 $\pm$ 0.45 | 24.64 $\pm$ 1.33 | 30.64 $\pm$ 0.67 | 40.63 $\pm$ 2.11 | 50.62 $\pm$ 1.33 |
| 9             | 13.98 $\pm$ 1.13           | 18.82 $\pm$ 1.45 | 23.67 $\pm$ 1.98 | 28.52 $\pm$ 1.76 | 33.37 $\pm$ 0.78 | 41.45 $\pm$ 1.46 | 49.53 $\pm$ 1.48 |
| 10            | 24.34 $\pm$ 1.32           | 33.89 $\pm$ 4.12 | 43.44 $\pm$ 0.34 | 52.98 $\pm$ 0.46 | 62.53 $\pm$ 1.78 | 78.44 $\pm$ 1.55 | 94.35 $\pm$ 1.97 |
| 11            | 18.00 $\pm$ 2.18           | 24.48 $\pm$ 0.86 | 30.97 $\pm$ 0.84 | 37.46 $\pm$ 0.57 | 43.95 $\pm$ 0.32 | 54.76 $\pm$ 0.56 | 65.57 $\pm$ 0.28 |
| 12            | 12.80 $\pm$ 3.62           | 18.91 $\pm$ 1.09 | 25.01 $\pm$ 1.43 | 31.12 $\pm$ 0.45 | 37.22 $\pm$ 1.36 | 47.40 $\pm$ 0.76 | 57.57 $\pm$ 0.93 |
| 13            | 25.33 $\pm$ 4.51           | 31.74 $\pm$ 0.74 | 38.16 $\pm$ 0.56 | 44.58 $\pm$ 1.44 | 51.00 $\pm$ 1.22 | 61.70 $\pm$ 1.33 | 72.39 $\pm$ 0.86 |
| 14            | 13.02 $\pm$ 2.22           | 18.97 $\pm$ 0.38 | 24.92 $\pm$ 0.86 | 30.87 $\pm$ 1.32 | 36.81 $\pm$ 0.87 | 46.73 $\pm$ 1.22 | 56.64 $\pm$ 1.45 |
| 15            | 16.16 $\pm$ 2.81           | 22.59 $\pm$ 0.48 | 29.01 $\pm$ 1.08 | 35.44 $\pm$ 0.98 | 41.86 $\pm$ 1.46 | 52.57 $\pm$ 1.92 | 63.28 $\pm$ 1.59 |
| Ascorbic acid | 11.75 $\pm$ 2.50           | 13.89 $\pm$ 0.98 | 20.45 $\pm$ 1.21 | 32.56 $\pm$ 1.43 | 42.45 $\pm$ 2.11 | 50.89 $\pm$ 0.67 | -----            |

**Table 3.** Percentage inhibition of Hydroxyl Radical using deoxyribose method.

| Compound      | Quantity ( $\mu\text{M}$ ) |                  |                  |                  |                  |                  |                  |
|---------------|----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|               | 3                          | 4.5              | 9                | 12               | 15               | 20               | 25               |
| 1             | 13.40 $\pm$ 1.12           | 17.63 $\pm$ 1.22 | 30.31 $\pm$ 2.34 | 38.77 $\pm$ 1.45 | 47.22 $\pm$ 0.67 | 61.31 $\pm$ 1.78 | 75.40 $\pm$ 0.97 |
| 2             | 17.56 $\pm$ 0.97           | 22.43 $\pm$ 2.11 | 37.04 $\pm$ 2.65 | 46.77 $\pm$ 1.67 | 56.51 $\pm$ 0.87 | 72.73 $\pm$ 1.43 | 88.96 $\pm$ 1.98 |
| 3             | 17.87 $\pm$ 0.44           | 23.14 $\pm$ 0.67 | 38.98 $\pm$ 0.76 | 49.53 $\pm$ 1.98 | 60.09 $\pm$ 0.56 | 77.68 $\pm$ 1.56 | 95.27 $\pm$ 0.66 |
| 4             | 14.22 $\pm$ 0.66           | 19.77 $\pm$ 0.56 | 36.41 $\pm$ 1.34 | 47.51 $\pm$ 0.99 | 58.60 $\pm$ 0.97 | 77.09 $\pm$ 1.67 | 95.59 $\pm$ 1.46 |
| 5             | 20.28 $\pm$ 0.46           | 23.57 $\pm$ 0.76 | 33.44 $\pm$ 3.12 | 40.02 $\pm$ 0.87 | 46.60 $\pm$ 1.23 | 57.57 $\pm$ 2.12 | 68.54 $\pm$ 1.87 |
| 6             | 14.47 $\pm$ 0.32           | 17.89 $\pm$ 0.98 | 28.13 $\pm$ 1.23 | 34.96 $\pm$ 1.45 | 41.79 $\pm$ 2.11 | 53.17 $\pm$ 2.23 | 64.56 $\pm$ 0.76 |
| 7             | 12.77 $\pm$ 0.45           | 16.92 $\pm$ 0.77 | 29.36 $\pm$ 1.98 | 37.66 $\pm$ 1.67 | 45.95 $\pm$ 0.87 | 59.78 $\pm$ 0.98 | 73.60 $\pm$ 2.43 |
| 8             | 8.79 $\pm$ 0.23            | 12.74 $\pm$ 1.76 | 24.58 $\pm$ 0.97 | 32.47 $\pm$ 2.56 | 40.37 $\pm$ 0.99 | 53.52 $\pm$ 0.87 | 66.68 $\pm$ 1.98 |
| 9             | 7.71 $\pm$ 1.43            | 11.28 $\pm$ 1.90 | 21.99 $\pm$ 0.66 | 29.13 $\pm$ 0.98 | 36.27 $\pm$ 0.56 | 48.17 $\pm$ 1.66 | 60.07 $\pm$ 0.65 |
| 10            | 28.13 $\pm$ 1.21           | 33.68 $\pm$ 0.76 | 50.33 $\pm$ 0.75 | 61.43 $\pm$ 0.67 | 72.52 $\pm$ 1.33 | 91.02 $\pm$ 1.79 | -----            |
| 11            | 20.79 $\pm$ 0.66           | 26.13 $\pm$ 0.98 | 42.12 $\pm$ 0.87 | 52.78 $\pm$ 0.68 | 63.44 $\pm$ 1.45 | 81.21 $\pm$ 2.01 | 98.98 $\pm$ 1.23 |
| 12            | 22.31 $\pm$ 0.49           | 26.13 $\pm$ 0.65 | 37.58 $\pm$ 1.76 | 45.22 $\pm$ 0.88 | 52.85 $\pm$ 0.87 | 65.58 $\pm$ 1.57 | 78.31 $\pm$ 1.44 |
| 13            | 26.05 $\pm$ 0.34           | 31.49 $\pm$ 0.87 | 47.81 $\pm$ 2.01 | 58.69 $\pm$ 1.02 | 69.56 $\pm$ 0.65 | 87.69 $\pm$ 1.22 | -----            |
| 14            | 18.68 $\pm$ 0.97           | 22.79 $\pm$ 0.43 | 35.12 $\pm$ 0.76 | 43.34 $\pm$ 1.67 | 51.56 $\pm$ 1.34 | 65.26 $\pm$ 2.12 | 78.95 $\pm$ 1.67 |
| 15            | 20.03 $\pm$ 0.67           | 24.41 $\pm$ 1.11 | 37.56 $\pm$ 0.33 | 46.33 $\pm$ 1.86 | 55.09 $\pm$ 1.66 | 69.70 $\pm$ 3.01 | 84.31 $\pm$ 1.98 |
| Ascorbic acid | 17.56 $\pm$ 0.78           | 20.46 $\pm$ 0.66 | 32.21 $\pm$ 1.23 | 45.57 $\pm$ 2.45 | 53.86 $\pm$ 0.98 | 75.86 $\pm$ 0.33 | -----            |

**Table 4:** Percentage inhibition of lipid peroxidation using thiobarbituric acid method.

| Compounds     | Quantity ( $\mu\text{M}$ ) |                  |                  |                  |                  |                  |                  |
|---------------|----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|               | 10                         | 15               | 20               | 30               | 45               | 50               | 75               |
| 1             | 15.69 $\pm$ 1.23           | 20.16 $\pm$ 2.18 | 24.64 $\pm$ 1.22 | 33.58 $\pm$ 0.56 | 46.99 $\pm$ 1.45 | 51.46 $\pm$ 1.24 | 73.82 $\pm$ 2.34 |
| 2             | 17.25 $\pm$ 1.87           | 22.17 $\pm$ 2.67 | 27.09 $\pm$ 1.45 | 36.93 $\pm$ .98  | 51.68 $\pm$ 1.67 | 56.60 $\pm$ 1.56 | 81.19 $\pm$ 0.99 |
| 3             | 22.39 $\pm$ 1.66           | 29.82 $\pm$ 1.23 | 37.26 $\pm$ 1.76 | 52.14 $\pm$ 1.24 | 74.45 $\pm$ 1.98 | 81.89 $\pm$ 1.67 | -----            |
| 4             | 20.38 $\pm$ 2.23           | 26.77 $\pm$ 0.97 | 33.16 $\pm$ 1.98 | 45.95 $\pm$ 1.87 | 65.13 $\pm$ 0.98 | 71.53 $\pm$ 2.78 | -----            |
| 5             | 14.34 $\pm$ 0.66           | 18.87 $\pm$ 0.67 | 23.40 $\pm$ 0.97 | 32.46 $\pm$ 1.90 | 46.06 $\pm$ 0.66 | 50.59 $\pm$ 3.01 | 73.24 $\pm$ 1.86 |
| 6             | 11.59 $\pm$ 0.76           | 16.44 $\pm$ 0.56 | 21.28 $\pm$ 0.76 | 30.98 $\pm$ 1.45 | 45.51 $\pm$ 0.54 | 50.36 $\pm$ 0.98 | 74.59 $\pm$ 1.34 |
| 7             | 14.97 $\pm$ 1.78           | 19.52 $\pm$ 0.93 | 24.07 $\pm$ 0.54 | 33.18 $\pm$ 2.12 | 46.84 $\pm$ 1.45 | 51.39 $\pm$ 1.22 | 74.16 $\pm$ 1.56 |
| 8             | 16.14 $\pm$ 1.98           | 20.41 $\pm$ 0.56 | 24.69 $\pm$ 0.65 | 33.23 $\pm$ 2.67 | 46.05 $\pm$ 1.67 | 50.32 $\pm$ 1.46 | 71.68 $\pm$ 1.48 |
| 9             | 15.49 $\pm$ 2.46           | 19.54 $\pm$ 1.22 | 23.59 $\pm$ 0.68 | 31.68 $\pm$ 0.94 | 43.82 $\pm$ 2.42 | 47.87 $\pm$ 1.87 | 68.10 $\pm$ 2.28 |
| 10            | 28.43 $\pm$ 0.56           | 38.88 $\pm$ 2.12 | 49.32 $\pm$ 0.89 | 70.21 $\pm$ 0.65 | -----            | -----            | -----            |
| 11            | 19.98 $\pm$ 0.98           | 28.19 $\pm$ 2.45 | 36.40 $\pm$ 1.56 | 52.82 $\pm$ 0.78 | 77.44 $\pm$ 0.99 | 85.65 $\pm$ 2.12 | -----            |
| 12            | 24.14 $\pm$ 0.63           | 30.66 $\pm$ 0.68 | 37.17 $\pm$ 1.98 | 50.21 $\pm$ 0.78 | 69.76 $\pm$ 0.68 | 76.27 $\pm$ 2.26 | -----            |
| 13            | 29.47 $\pm$ 0.89           | 38.95 $\pm$ 0.99 | 48.43 $\pm$ 1.77 | 67.38 $\pm$ 1.45 | 95.82 $\pm$ 1.67 | -----            | -----            |
| 14            | 20.64 $\pm$ 1.55           | 27.53 $\pm$ 1.56 | 34.42 $\pm$ 2.12 | 48.19 $\pm$ 2.34 | 6.86 $\pm$ 1.89  | 75.74 $\pm$ 2.56 | -----            |
| 15            | 24.05 $\pm$ 2.12           | 30.97 $\pm$ 2.44 | 37.89 $\pm$ 2.45 | 51.73 $\pm$ 0.66 | 72.49 $\pm$ 2.19 | 79.86 $\pm$ 2.34 | -----            |
| Ascorbic acid | 20.30 $\pm$ 0.78           | 28.89 $\pm$ 0.67 | 37.15 $\pm$ 0.89 | 50.15 $\pm$ 2.08 | 71.26 $\pm$ 2.89 | 82.85 $\pm$ 1.56 | -----            |

**Table 5:** ABTS<sup>+</sup> cation radical scavenging activity of test compounds and ascorbic acid.

| Compounds     | Quantity ( $\mu\text{M}$ ) |                  |                  |                  |                  |                  |                  |                  |
|---------------|----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|               | 0.75                       | 1.5              | 3                | 5                | 7.5              | 10               | 15               | 20               |
| 1             | 17.67 $\pm$ 1.25           | 20.85 $\pm$ 0.87 | 27.19 $\pm$ 1.23 | 35.65 $\pm$ 0.76 | 46.22 $\pm$ 1.23 | 56.79 $\pm$ 0.99 | 77.94 $\pm$ 1.90 | -----            |
| 2             | 17.65 $\pm$ 2.12           | 20.91 $\pm$ 0.76 | 27.44 $\pm$ 1.65 | 36.15 $\pm$ 0.45 | 47.04 $\pm$ 1.98 | 57.93 $\pm$ 0.67 | 79.70 $\pm$ 1.56 | -----            |
| 3             | 18.86 $\pm$ 1.56           | 22.39 $\pm$ 1.78 | 29.45 $\pm$ 0.98 | 38.88 $\pm$ 0.43 | 50.65 $\pm$ 0.76 | 62.43 $\pm$ 0.65 | 85.98 $\pm$ 0.99 | -----            |
| 4             | 13.83 $\pm$ 0.98           | 17.54 $\pm$ 1.89 | 24.97 $\pm$ 0.78 | 34.88 $\pm$ 1.95 | 47.26 $\pm$ 0.45 | 59.64 $\pm$ 0.34 | 84.41 $\pm$ 1.45 | -----            |
| 5             | 18.92 $\pm$ 0.76           | 21.27 $\pm$ 2.04 | 25.97 $\pm$ 0.95 | 32.24 $\pm$ 1.67 | 40.07 $\pm$ 1.67 | 47.91 $\pm$ 0.98 | 63.58 $\pm$ 1.78 | 79.25 $\pm$ 2.11 |
| 6             | 10.52 $\pm$ 0.89           | 13.43 $\pm$ 1.90 | 19.26 $\pm$ 1.56 | 27.03 $\pm$ 0.65 | 36.74 $\pm$ 1.09 | 46.46 $\pm$ 1.89 | 65.88 $\pm$ 1.66 | 85.31 $\pm$ 1.56 |
| 7             | 15.70 $\pm$ 0.56           | 19.02 $\pm$ 1.56 | 25.64 $\pm$ 2.67 | 34.48 $\pm$ 0.43 | 45.52 $\pm$ 2.09 | 56.57 $\pm$ 1.67 | 78.66 $\pm$ 1.45 | -----            |
| 8             | 11.71 $\pm$ 0.66           | 14.14 $\pm$ 1.89 | 18.99 $\pm$ 0.63 | 25.47 $\pm$ 0.65 | 33.55 $\pm$ 2.56 | 41.64 $\pm$ 1.80 | 57.82 $\pm$ 0.78 | 74.00 $\pm$ 1.67 |
| 9             | 15.13 $\pm$ 0.99           | 17.22 $\pm$ 2.11 | 21.40 $\pm$ 0.91 | 26.97 $\pm$ 0.87 | 33.93 $\pm$ 2.11 | 40.90 $\pm$ 1.78 | 54.82 $\pm$ 1.02 | 68.75 $\pm$ 1.89 |
| 10            | 20.70 $\pm$ 0.45           | 25.65 $\pm$ 2.02 | 35.54 $\pm$ 1.67 | 48.73 $\pm$ 2.12 | 65.21 $\pm$ 0.45 | 81.70 $\pm$ 2.12 | -----            | -----            |
| 11            | 12.51 $\pm$ 1.90           | 16.74 $\pm$ 1.56 | 25.19 $\pm$ 0.65 | 36.47 $\pm$ 2.24 | 50.56 $\pm$ 0.56 | 64.65 $\pm$ 2.45 | 92.83 $\pm$ 1.67 | -----            |
| 12            | 15.72 $\pm$ 1.56           | 18.07 $\pm$ 1.87 | 22.76 $\pm$ 0.43 | 29.02 $\pm$ 0.56 | 36.84 $\pm$ 0.78 | 44.66 $\pm$ 1.11 | 60.30 $\pm$ 1.90 | 75.95 $\pm$ 1.99 |
| 13            | 22.00 $\pm$ 2.13           | 25.89 $\pm$ 2.02 | 33.67 $\pm$ 1.34 | 44.05 $\pm$ 1.45 | 57.01 $\pm$ 0.92 | 69.98 $\pm$ 1.77 | 95.91 $\pm$ 1.76 | -----            |
| 14            | 19.30 $\pm$ 0.87           | 21.28 $\pm$ 2.32 | 25.23 $\pm$ 2.89 | 30.51 $\pm$ 1.98 | 37.11 $\pm$ 1.98 | 43.70 $\pm$ 0.87 | 56.90 $\pm$ 0.95 | 70.09 $\pm$ 1.61 |
| 15            | 7.40 $\pm$ 0.65            | 10.39 $\pm$ 0.45 | 16.38 $\pm$ 2.11 | 24.37 $\pm$ 1.49 | 34.35 $\pm$ 1.56 | 44.34 $\pm$ 0.66 | 64.31 $\pm$ 1.87 | 84.28 $\pm$ 0.92 |
| Ascorbic acid | 12.35 $\pm$ 0.59           | 23.46 $\pm$ 0.44 | 27.86 $\pm$ 0.65 | 35.58 $\pm$ 3.12 | 46.89 $\pm$ 1.56 | 57.53 $\pm$ 0.78 | 68.68 $\pm$ 1.66 | -----            |

### Discussion

The antioxidant activities of the above mentioned compounds (1-15) was determined through their abilities to scavenge the superoxide, hydroxyl and ABTS radical, in addition to these abilities to inhibit membrane lipid peroxidation. The ABTS<sup>+</sup> radical cation is generated from the over-night reaction of ABTS with potassium persulfate, followed by dilution with ethanol. The decolorization of the ABTS<sup>+</sup> radical cation also reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate this radical species. The results given in Tables 1-5 illustrated that compounds 10 and 13 possessing the trimethoxy substitution on phenyl ring at position 5 of the 2-pyrazolines exhibit maximum activity, in fact more than that of the standard drug ascorbic acid. Compounds 11, 3, 4, 2 and 1 having dimethyl amino group at position 4' of the phenyl ring at position 5 of the 2-pyrazolines also showed good activity. Among these compounds 3, 4 and 2 which possess apart from dimethyl amino group at 4' position also have an electron releasing substituent either at position 2" or 4" of the phenyl ring at position-2 of the 2-pyrazolines showed more activity than compound 1 which does not possess such substituents. Interestingly, it is also observed that compounds 5-9, 12, 14 which possess neither a dimethyl amino group nor a trimethoxy substitution on the phenyl ring at position 5 of the 2-pyrazolines showed less activity. This revealed that an electron releasing group at position 4' of the phenyl ring is very much essential for all these 2-pyrazolines to show significant activity.

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