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MICROWAVE IRRADIATION SYNTHESIS AND ANTIOXIDANT ACTIVITY OF ISATIN-OXADIAZOLE DERIVATIVES

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ABSTRACT

Based on the literature review, we have found that Isatin- Oxadiazole individual derivatives are exhibiting different activities like anti oxidant, anti viral, anti microbial and anti convulsant and no one have reported the synthesis of Isatin- Oxadiazole hybrids. Among the series compound S 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-chloro indolin 2-one (IVb), 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-bromo indolin 2-one(IVe) showed most significant antioxidant activity by Hydrogen peroxide scavenging assay.

INTRODUCTION:

Heterocycles form the largest of classical divisions of organic chemistry and are of immense importance both biologically and industrially. The majority of pharmaceuticals and agrochemicals are heterocyclic while countless additives and modifiers used in industrial applications ranging from cosmetics, reprography, information storage and plastics are heterocyclic molecules. One of the striking structural features inherent to heterocycles, which continue to be exploited to great advantage by the drug industry, lies in their ability to manifest substituents around a core scaffold in defined three dimensional representations. For more than a century, heterocycles have constituted one the largest areas of research in organic chemistry. They have contributed to the development of society through health care and industrial point of view as well as in understanding life processes and thus improving the quality of life. Various compounds such as nucleic acids, alkaloids, antibiotics, essential amino acids, vitamins, hemoglobin, hormones and a large number of synthetic drugs contain heterocyclic ring systems. Among the wide variety of heterocyclic compounds that have been explored for developing pharmaceutically important molecules, isatin have played an important role.

CHEMISTRY OF ISATIN

Isatin (1H-indole-2, 3-Dione) (I) was first discovered by Erdmann[1] and Laurent[2] in 1841, independently as a product from oxidation of indigo by nitric and chromic acids. It is a unique molecule possessing both amide and ketocarbonyl groups. Apart from this, it has an active hydrogen atom attached to nitrogen (or oxygen) and an aromatic ring which should substitute at 5- and 7-positions. It exists in a tautomeric form (II) and these functional characteristics play an important role in governing the various reactions of the molecule. The C-3 carbonyl group of isatin is strongly electrophilic. As a result, isatins are readily involved in condensation and addition reactions with carbanion type nucleophiles

into 3-substituted oxindoles[3]. In general, there are three possibilities during condensation reactions. Both the α, β-carbonyl groups, having varying reactivity are involved, Ring cleavage takes place and Ring expansion occurs. A general observation has been that the nature of final product always depends on the experimental conditions and substituents at nitrogen atom, which may affect the electron density at, α and β carbonyl carbon atoms respectively.

OXADIAZOLES:

Oxadiazole is a heterocyclic aromatic chemical compound containing an oxygen and two nitrogen atoms in a five membered ring. There are 4 isomers, 1,2,4-oxadiazole, 1,2,3-oxadiazole, 1,2,5-oxadiazole and 1,3,4-oxadiazole. 1,3,4-oxadiazole is better known and more widely studied because of its many important chemical and biological properties. Among heterocyclic compounds 1,3,4-oxadiazole has become an important construction motif for the development of new drugs.

EXPERIMENT WORK

Step: 1

Synthesis of Isatin Carbohydrazone:

A mixture of Isatin(0.1M), Carbohydrazide(0.1M) are dissolved in Ethanol, then add 2-3 drops of Glacial acetic acid. The progress of the reaction was monitored by TLC. refluxed for 90 minutes/ MWI 10 Min. The reaction mixture was filtered by using whatmann filterpaper and funnel. And dried product was purified by using ethyl acetate.

Step: 2

Synthesis of 3-(2-(5-Mercapto-1, 3, 4-Oxadiazol-2-YL) Hydrazono) Indolin-2-One:

Weigh accurately 0.028M of Isatin carbohydrazone and 0.028M KOH and dissolved in Ethanol, when it is in heating conditions, add 35mmol(0.035M) of carbon disulphide and put it reflux for 2-3 hrs/ MWI 10-12 Min, until the evaluation of hydrogen sulphide ceased. The reaction mixture was cooled to room temperature and poured into ice cold water (100ml). it was neutralized with dilute hydrochloric acid. The precipitated solid was filtered, washed with water and dried product was recrystallizing from ethanol.

DERIVATIVES: 5-Chloro Isatin,5-Methoxy Isatin, 4,6-Dichloro isatin and 5-Br isatin are the starting materials which are used for the respective 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-chloro indolin 2-one, 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-methoxy indolin 2-one, 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-4,6 di chloro indolin 2-one, 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl) hydrazono)-5-bromo indolin 2-one.

PHARMACOLOGICAL EVALUATION

Anti-oxidant activity

In view of varied biological and pharmacological importance of different series of pyrimidine derivatives, the synthesized compounds were screened for anti-oxidant activity. Antioxidants are intimately involved in the prevention of free radicals, which cause DNA damage. By studying various methods, the following methods describe anti-oxidant activity. Hydrogen peroxide scavenging method and Nitric oxide scavenging method

Hydrogen peroxide scavenging method

Materials

Hydrogen Peroxide, Phosphate Buffer, Ascorbic Acid

Method

The synthesized compounds scavenged to hydrogen peroxide was determined according to the method of Ruch et al (1989). A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilledwater were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance ofhydrogen peroxide at 230 nm was determined 10 minutes later against a blanksolution containing the phosphate buffer without hydrogen peroxide. The percentageof hydrogen peroxide scavenging of both C. monogyna extracts and standardcompounds were calculated:

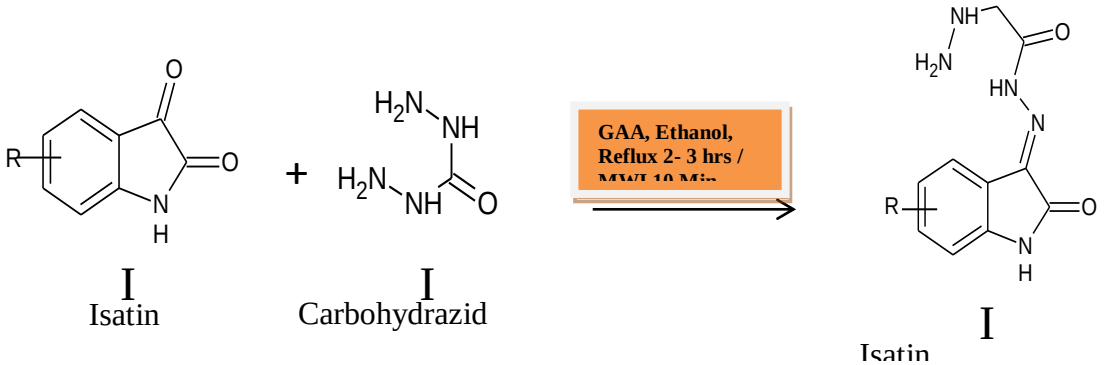
% Scavenged [H_2O_2] = [(A_c – As/Ac] x 100

Where AC is the absorbance of the control and AS is the absorbance in the presence of the test compunds or standards.

Preparation of phosphate buffer (pH7.4)

6.8gms of KH_2PO_4(potassium dihydrogen phosphate)+1.5gms of NaOH(sodium hydroxide) weredissolved in 1000ml of Millipore water.

SCHEME

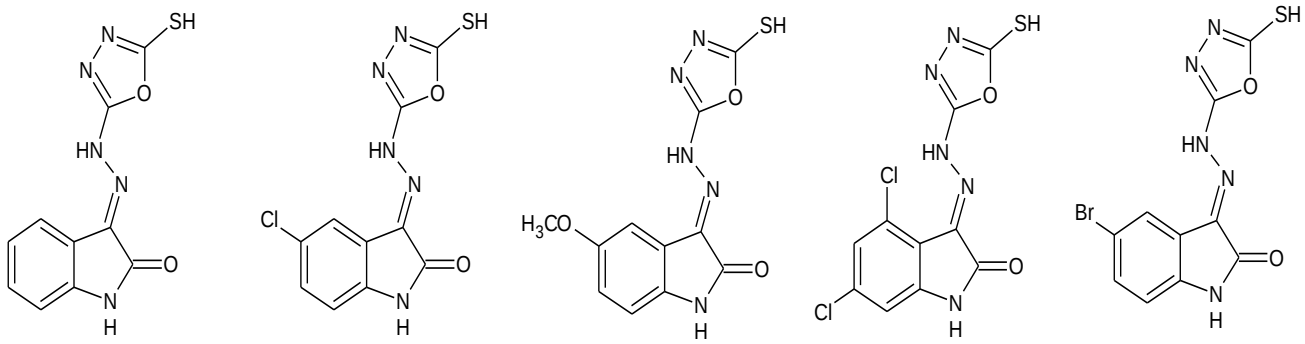


I
Isatin

I
Carbohydrazid

I
Isatin

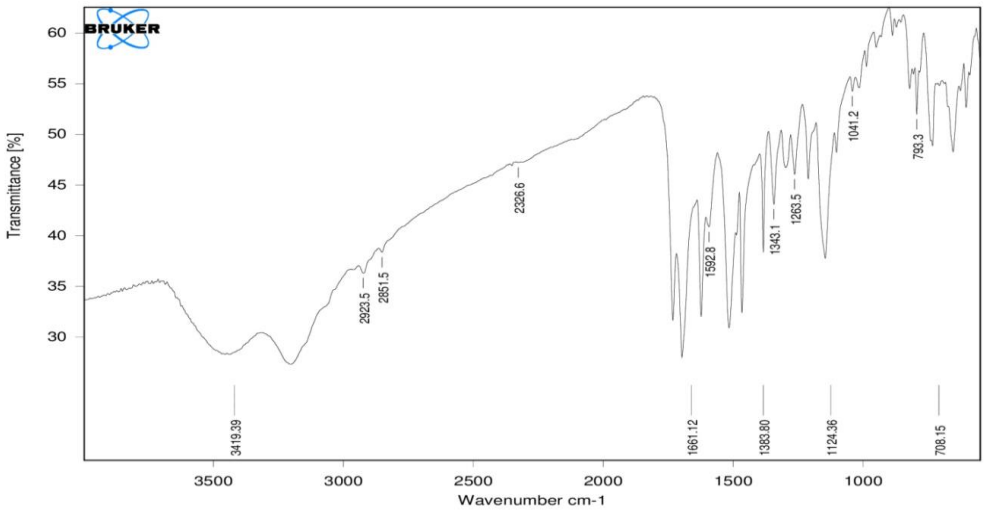
R – 5-H (I_a)
R – 5-Cl (I_b)
R – 5-OCH_3 (I_c)
R – 4, 6-dichloro (I_d)
R – 5-Br (I_e)

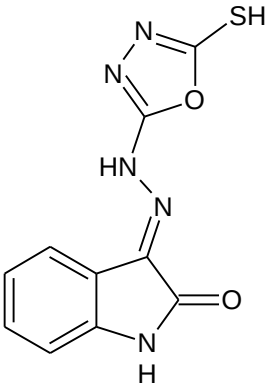


IV_a IV_b IV_c IV_d IV_e

S.no		Derivatives	Molecular Formula	Molecular Weight	Melting Point
1	IV a	R-5H	$C_{10}H_7N_5O_2S_1$	260	205-208
2	IV b	R-5Cl	$C_{10}H_6N_5O_2S_1Cl_1$	295	206-208
3	IV c	R-5-OCH_3	$C_{10}H_9N_5O_3S_1$	291	204-207
4	IV d	R-4-Cl,R1-6-Cl	$C_{10}H_5N_5O_2S_1Cl_2$	329	207-209
5	IV e	R-5Br	$C_{10}H_6N_5O_2S_1Br_1$	340	205-207

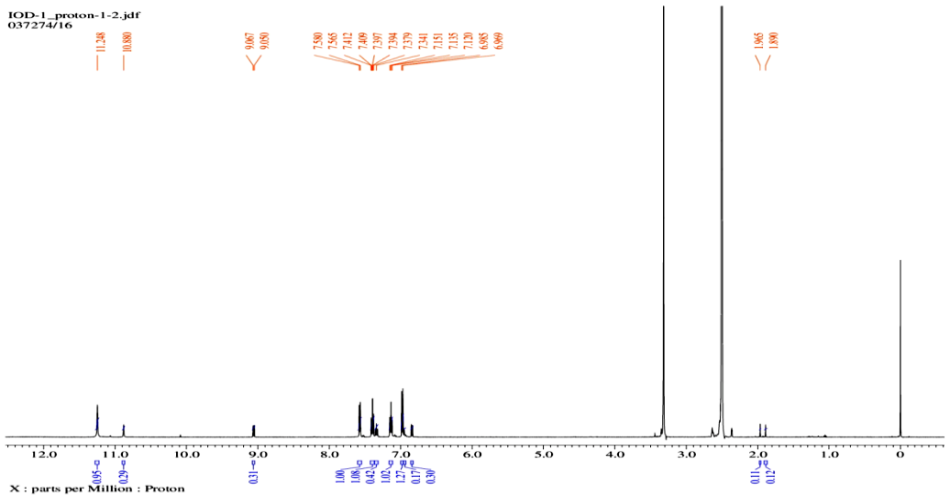
FT-IR SPECTRAM

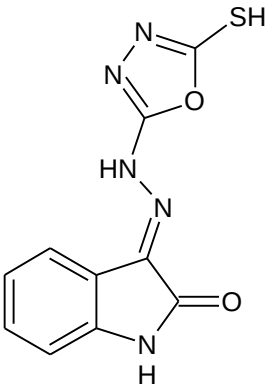




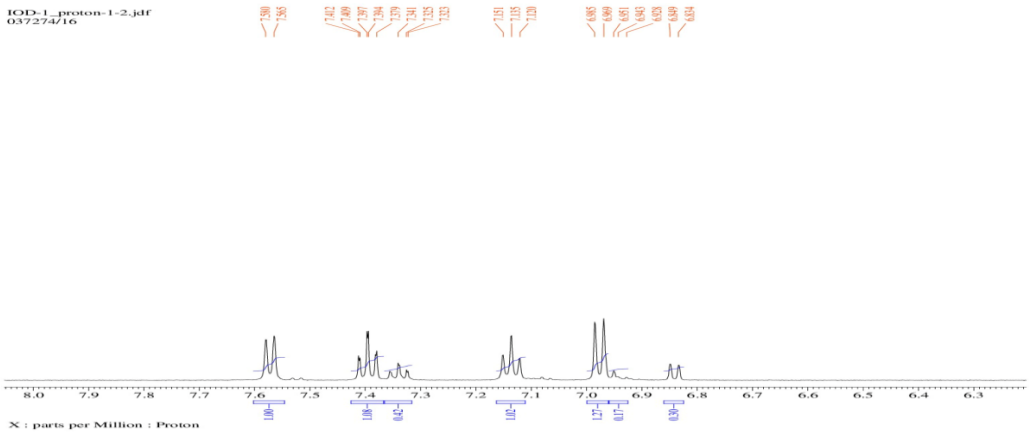
3-(2-(5 –mercapto-1, 3, 4-oxadiazole-2-yl)hydrazono)indolin-2-one(IVa)

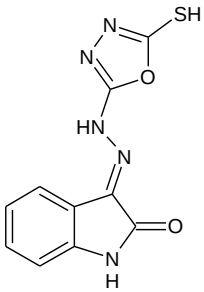
[1]HNMR-SPECTRAM





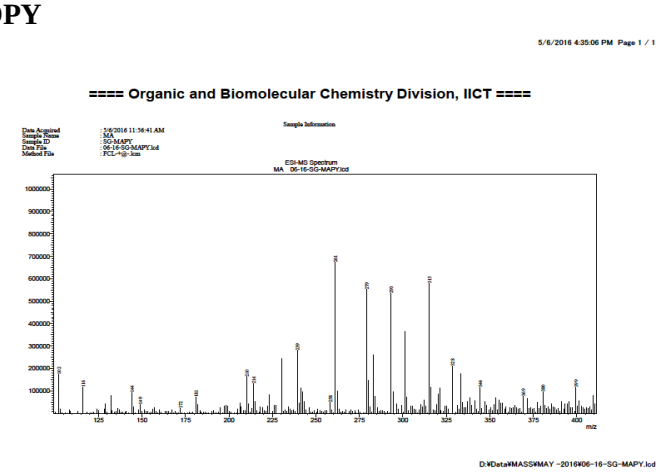
3-(2-(5 –mercapto-1,3,4-oxadiazole-2-yl)hydrazono)indolin-2-one(IVa)

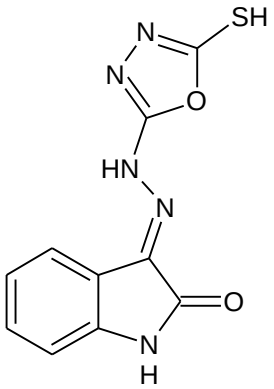




3-(2-(5 –mercapto-1, 3, 4-oxadiazole-2-yl)hydrazono)indolin-2-one(IVa)

MASS SPECTROSCOPY





3-(2-(5 –mercapto-1, 3, 4-oxadiazole-2-yl)hydrazono)indolin-2-one(IVa)

- ✓ **FT-IR SPECTUM:**
  From the FT-IR spectrum, we have found that the peak at 3419.39cm^{-1} is due to the presence of N-H stretching, and the peak at 1661.12cm^{-1} is due to the presence of C=O stretching**.**
- ✓ **^{1}HNMR SPECTRUM:**
  From the ^{1}HNMR spectrum, we confirmed the presence of number of protons the peaks absorbed between 6.8-7.6 indicate the presence of aromatic protons.
- ✓ **MASS SPECTRUM:**
- ✓ From the Mass spectrum, we have found that the molecular ion peak appears at 261(M+1) there confirms the most abundant peak confirms the formation of final compound**.**

Preparation of 40mM Hydrogen Peroxide

0.228ml of H_2O_2 is dissolved in 50ml of phosphate buffer (pH7.4)

Preparation of standard solution (Ascorbic Acid)

10mg of ascorbic acid is dissolved in 10ml of phosphate buffer (pH7.4)

Prepation of test solution (synthesized compound)

10mg of sample is dissolved in 10ml of phosphate buffer (pH7.4)

Nitric oxide scavenging assay:

Materials

Sodium nitroprusside, phosphate buffered saline, Griess reagent, methanol

Method

Nitric oxide scavenging activity was measured spectrophotometrically. Sodium nitroprusside (3.0ml of 5mM) in phosphate buffered saline pH 7.4, was mixed with differentconcentrations of the compound prepared in methanol and incubated at 25 °C for 30 min. A control without the test compound, with an equivalent amount of methanol, was taken. After 30 min, 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1- naphthylethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard.

RESULTS AND DISCUSSION:

The preliminary studies on possible pharmacological activities of isatin-oxadiazole derivatives have generated some useful data. The anti-oxidant activity of the synthesized compounds Iva-e were evaluated by in vitro hydrogen scavenging assay method.The results are recorded in Table 2. Among the series **(Iv**a-e) 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-chloro indolin 2-one, 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-bromo indolin 2-one showed most significant activity with Ic_{50} values of 3.74±0.303,3.72±0.043. Compounds 3-(2-(5 –mercapto-1,3,4-oxadiazole-2-yl)hydrazono)indolin-2-one**,** 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl) hydrazono) - 4,6 di chloro indolin 2-one showed good antioxidant activity with Ic_{50} values of 3.68±0.0307,3.65±0.0204,3. Compound 3-(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-methoxy indolin 2-one, showed less antioxidant

activity with Ic_{50} value of 3.55±0.026. Among the series compound of 3-(2-(5-mercapto-1,3,4-oxadiazole-2-yl) hydrazono)indolin-2-one showed significant antioxidant activity by Ic_{50} value of 3.77±0.030. Compound 3-(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-methoxy indolin 2-one, shows good antioxidant activity by Ic_{50} value of 3.68±0.038. Compounds **(IVb),(IVd),(IV-e),** showed less antioxidant activity

Five new Isatin- oxadiazole derivatives have been synthesized by using appropriate synthetic route. Final compounds are characterized by physical & spectral data (FT-IR, NMR,Mass) From the FT-IR spectrum, we have found that the peak at 3419.39cm^{-1} is due to the presence of N-H stretching, and the peak at 1661.12cm^{-1} is due to the presence of C=O stretching**.** From the ^{1}HNMR spectrum, we confirmed the presence of number of protons the peaks absorbed between 6.8-7.6 indicate the presence of aromatic protons. From the Mass spectrum, we have found that the molecular ion peak appears at 261(M+1) there confirms the most abundant peak confirms the formation of final compound**.**

Activity: All the synthesized compounds are evaluated for antioxidant activity by Hydrogen peroxide scavenging assay & Nitric oxide scavenging assay, the results are clearly reveals that most of the compounds showing good antioxidant activity as compared with the standard drug(Ascorbic acid).

CONCLUSION:

Isatin-Oxadiazole derivatives have been synthesized & their structures are elucidated by physical& spectral data. From the FT-IR spectrum, we have found that the peak at 3419.39cm^{-1} is due to the presence of N-H stretching, and the peak at 1661.12cm^{-1} is due to the presence of C=O stretching**.** From the ^{1}HNMR spectrum, we confirmed the presence of number of protons the peaks absorbed between 6.8-7.6 indicate the presence of aromatic protons. From the Mass spectrum, we have found that the molecular ion peak appears at 261(M+1) there confirms the most abundant peak confirms the formation of final compound**.** The compounds which are having electron withdrawing groups are showing activity compared to all other tested compounds**.**

- The studies about synthetic work have been carried out by standard procedures. All the synthesized compounds are evaluated for antioxidant activity by Hydrogen peroxide scavenging assay & Nitric oxide scavenging assay, the results are clearly reveals that most of the compounds showing good antioxidant activity as compared with the standard drug (Ascorbic acid).
- Among the series compound 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-chloro indolin 2-one(IVb), have shown good yield.
- All the compounds are evaluated for antioxidant activity by using standard protocols.
- Among the series compoundS 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-chloro indolin 2-one(IVb), 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-bromo indolin 2-one(IVe) showed most significant antioxidant activity by Hydrogen peroxide scavenging assay.
- Compounds 3-(2-(5 –mercapto-1,3,4-oxadiazole-2-yl)hydrazono)indolin-2-one**(IVa),** 3,(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-4,6 di chloro indolin 2-one(IVd) showed good antioxidant activity in the series by Hydrogen peroxide scavenging assay.
- Among the series compound of 3-(2-(5- mercapto-1,3,4-oxadiazole-2-yl)hydrazono)indolin-2-one(IVa) showed significant antioxidant activity by Nitric oxide scavenging assay.
- Compound 3-(2,(5-mercapto-1,3,4-oxadiazole-2-yl)hydrazono)-5-methoxy indolin 2-one (IVc) showed good antioxidant activity by Nitric oxide scavenging method.
- The compound **(R=Cl)** and **(R=H**have shown most significant antioxidant activity by Nitric oxide scavenging method and Hydrogen peroxide scavenging method and owing to its Strong activating electron donating nature.

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