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Syntheses and Antioxidant Screening of Pyrazole-4-Carboxaldehyde Derivatives

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ABSTRACT

Nine new derivatives of pyrazole-4-carboxaldehydes **(Va-i)** have been synthesized by acetic acid mediated condensation of different aromatic ketones with phenylhydrazines in ethyl alcohol to afford different phenylhydrazones. Phenylhydrazones so prepared were further allowed to react with two moles of DMF-$POCl_3$ adduct (Vilsmeier Haack reagent) in the DMF at 60-70°C for 6 hours with formation of immonium perchlorate. Introduction of phenyl ring at first & third position of pyrazole may increase the antioxidant activity. The participation of the C=C bond is important in stabilizing the antioxidant radical by resonance. Introduction of electron releasing groups on phenyl rings attached to heterocycles increase the electron donating capacity of antioxidants. Further more on alkaline hydrolyses (NaOH) they afforded different pyrazole-4-carboxaldehyde derivatives. The structures of synthesized compounds have been characterized on the basis of IR, 1H-NMR, ESI-MS and elemental analysis. All the synthesized compounds were screened for antioxidant activity. In order to neutralizing the threat of free radicals to the tissues and cells, body enzymes take participate include: glutathione peroxidise (GSH), superoxide dismutase (SOD) and catalase Antioxidants may intervene with these free radicals at different levels in the oxidative process. In FRAP assay, increased absorbance of the compounds with concentration indicates increased reducing power. Compounds with higher concentrations showed a higher reducing power. The reducing power showed good linear relation (R^2) in both standard as well as compounds. These results clearly reveal that compounds have antioxidant activity.

KEYWORDS: 1-H-pyrazole-4-carboxaldehyde; Pyrazole aldehyde derivatives; NMR; Mass; IR spectroscopy; Elemental analysis

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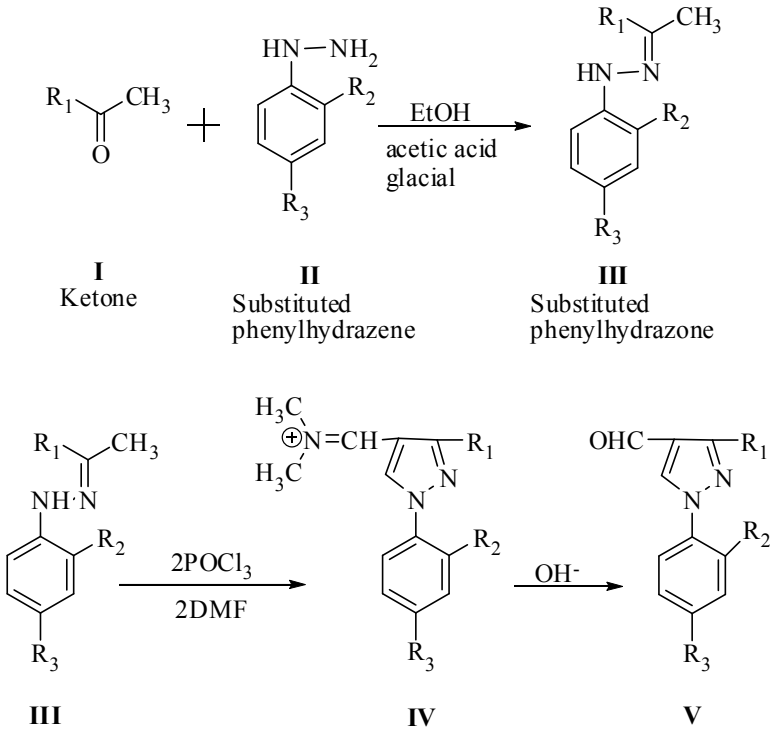
INTRODUCTION

Overall cell health depends on the balance between formation and elimination of free radicals. Free radicals, normally, continuously generated in the living cell, in low amounts by the transfer of one electron to an oxygen molecule during respiration chain and cellular immunization reactions and are therefore needed for the normal redox-signaling and self-defence of the host. Still, superoxide anion (O_2^-) and hydroxyl radical ($OH^\bullet$), in increased concentrations, can induce oxidative stress and cellular damage by altering the biological activities of lipids, proteins, DNA and carbohydrates, even to cellular death. ROS are associated with incidence of heart diseases, thrombosis, hypertension, Alzheimer's and Parkinson's diseases and cancer over the radical induced DNA double-strain breaks[1]. In order to neutralizing the threat of free radicals to the tissues and cells, body enzymes include: glutathione peroxidise (GSH), superoxide dismutase (SOD) and catalase [2, 3, 4, 5, 6]. Antioxidants may intervene with these free radicals at different levels in the oxidative process. The term Pyrazole was given by Ludwig Knorr in 1883 is a unique template that is associated with several biological activities. Pyrazole derivatives are of interest principally for antioxidant properties due to the presence of conjugated π-system, which delocalize after donation of hydrogen atom and stabilize the antioxidant molecule; activity is also related to the concentration and type of substituent present [7].

MATERIAL AND METHODS

The reagent grade chemicals required were obtained from Hi-media Chem. Ltd, SD-Fine Ltd. and Sigma Aldrich Pvt. Ltd and were used as such. Melting points were determined using open capillary tube melting point apparatus and are uncorrected. Reaction progress was monitored by performing thin layer chromatography on silica gel G plates, using iodine vapours and UV chamber as visualizing agents. After physical characterization (Table 2), the compounds were subjected to spectral analysis. Proton Nuclear Magnetic resonance spectra were recorded on Bruker WM-300 (at 300 MHz) spectrometer and chemical shifts are reported in parts per million (δ value) from TMS (δ 0 ppm for ^{1}H NMR) as an internal standard. Coupling constant are given in Hertz. Mass spectra were recorded on a JEOL-SX-102 instrument using ESI. Infrared spectra were taken on Shimadzu-700 spectrometer.

9 derivatives of pyrazole-4-carboxaldehyde (**V**) (Table 1) were obtained *via* the Vilsmeier-Haack reaction of the appropriate phenylhydrazones (**III**), derived from the reaction of ketones (**I**) with phenylhydrazines (**II**) (Scheme 1) [8].



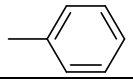
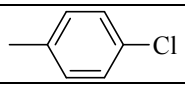
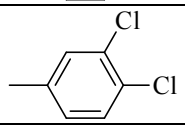
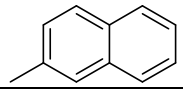
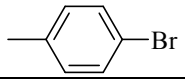
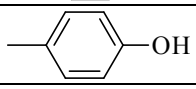
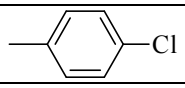
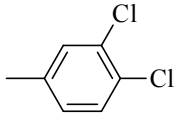
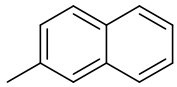
Scheme 1. Synthesis of pyrazole-4-carboxaldehydes

EXPERIMENTAL WORK

A mixture of 0.04 mol of ketones **(I)** and 0.04 mol of phenylhydrazines **(II)** with 60 ml of ethanol and few drops of glacial acetic acid were heated on water bath for 30 minutes. Filtered the cold reaction mixture; washed the solid with dilute HCl followed by 12 ml of cold rectified spirit. Recrystallized from ethanol and thus obtained a pure phenylhydrazone **(III)**. The progress of reaction was monitored by TLC using hexane and ethanol (90:10) [9,10].

Synthesis of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde **(Va-i)** was carried out by the application of two moles of cold solution of Vilsmeier-Haack (VH) reagent (DMF-$POCl_3$ adduct) in DMF with substituted phenylhydrazones **(IIIa-i)**. The reaction mixture was stirred at 70-80°C for 5-6 hours with formation of the immonium perchlorate **(IV)** and it was cooled to room temperature then poured into cold water and a saturated solution of sodium bicarbonate was added to neutralize the mixture and the solid obtained filtered followed by washing with water. The progress of reaction was monitored by TLC using hexane and ethanol (90:10) [11, 12].

Table 1: Compound and its reactive groups

Compound code	-R_1	-R_2	-R_3
(Va)		H	H
(Vb)		H	H
(Vc)		H	H
(Vd)		H	H
(Ve)		H	H
(Vf)		H	H
(Vg)		NO_2	NO_2
(Vh)		NO_2	NO_2
(Vi)		NO_2	NO_2

1-phenyl-2-(1-phenylethylidene) hydrazine (IIIa)

IR (KBr) cm^{-1}: 3018.39 (Aromatic =C-H), 1598.88 and 1444.58 (Aromatic C=C), 688.54 (Aromatic C=C-H bending), 1363.22 (Alkane CH_3), 1644.58 (Imine C=N), 3302.05 (N-H stretching), 1487.01 (N-H bending), 1363.22 (-C-N). MS (ESI): 210.5 (M^+).

1,3-diphenyl-1H-pyrazole-4-carbaldehyde (Va)

IR (KBr) cm^{-1}: 3060.82 (Aromatic C-H), 1600.98 and 1450.37 (Aromatic C=C), 697.81 (Aromatic C=C-H bending), 1702.12 and 1656.63 (C=C-CHO). MS (ESI): 248.4 (M^+). ^{1}H-NMR (δ/$CDCl_3$): δ 7.453-7.311 (t, 2H), δ 7.987-7.851 (t, 4H), δ 8.501-8.497 (d, 2H), δ 8.734-8.693 (d, 2H), δ 9.046 (s, 1H), δ 9.458 (s, 1H).

1-(1-(4-chlorophenyl)ethylidene)-2-phenylhydrazine (IIIb)

IR (KBr) cm^{-1}: 3069.40 (Aromatic =C-H), 1598.88 and 1444.58 (Aromatic C=C), 692.40 (Aromatic C=C-H bending), 1394.44 (Alkane CH_3), 1652.44 (Imine C=N), 3340.48 (N-H stretching), 1521.86 (N-H bending), 1004.84 (-C-N), 1060.13 (Aromatic-Cl). MS (ESI): 244.8 (M^+).

3-(4-chlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (Vb)

IR (KBr) cm^{-1}: 3051.58 (Aromatic C-H), 1601.88 and 1486.61 (Aromatic C=C), 686.61 (Aromatic C=C-H bending), 1672.17 and 1634.61 (C=C-CHO), 1091.63 (Aromatic-Cl). MS (ESI): 282.6 (M^+). ^{1}H-NMR (δ/$CDCl_3$): δ 7.675-7.598 (t, 1H), δ 7.949-7.894 (d, 2H), δ 8.296-8.137 (t, 2H), δ 8.548-8.499 (d, 2H), δ 8.726 (s, 1H), δ 8.953-8.915 (d, 2H), δ 9.552 (s, 1H)

1-(1-(3,4-dichlorophenyl)ethylidene)-2-phenylhydrazine (IIIc)

IR (KBr) cm^{-1}: 3050.39 (Aromatic =C-H), 1588.88 and 1448.10 (Aromatic C=C), 708.54 (Aromatic C=C-H bending), 1392.26 (Alkane CH_3), 1668.56 (Imine C=N), 3424.30 (N-H stretching), 1480.02 (N-H bending), 1392.26 (-C-N), 1071.76 (Aromatic-Cl). MS (ESI): 279.5 (M^+).

3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (Vc)

IR (KBr) cm^{-1}: 2925.81 (Aromatic C-H), 1602.81 and 1440.73 (Aromatic C=C), 688.54 (Aromatic C=C-H bending), 1681.81 and 1625.80 (C=C-CHO), 1147.27 (Aromatic-Cl). MS (ESI): 317.0 (M^+). ^{1}H-NMR (δ/$CDCl_3$): δ 7.702-7.594 (t, 1H), δ 7.953-7.922 (d, 1H), δ 8.227-8.124 (t, 2H), δ 8.398-8.356 (d, 2H), δ 8.594 (s, 1H), δ 8.801-8.782 (d, 1H), δ 9.069 (s, 1H), δ 9.498 (s, 1H).

1-(1-(naphthalen-2-yl)ethylidene)-2-phenylhydrazine (IIId)

IR (KBr) cm^{-1}: 3042.47 (Aromatic =C-H), 1602.54 and 1449.58 (Aromatic C=C), 642.12 (Aromatic C=C-H bending), 1374.69 (Alkane CH_3), 1782.04 (Imine C=N), 3345.05 (N-H stretching), 1548.19 (N-H bending), 1349.79 (-C-N). MS (ESI): 260.2 (M^+).

3-(naphthalen-2-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde (Vd)

IR (KBr) cm^{-1}: 3058.89 (Aromatic C-H), 1600.28 and 1465.80 (Aromatic C=C), 869.84 (Aromatic C=C-H bending), 1676.03 and 1625.88 (C=C-CHO). MS (ESI): 298.1 (M^+). ^{1}H-NMR (δ/$CDCl_3$): δ 7.394-7.309 (t, 1H), δ 7.689-7.604 (t, 4H), δ 7.932-7.902 (d, 2H), δ 8.115 (s, 1H), δ 8.400-8.387 (d, 2H), δ 8.626-8.612 (d, 2H), δ 9.018 (s, 1H), δ 9.497 (s, 1H).

1-(1-(4-bromophenyl)ethylidene)-2-phenylhydrazine (IIIe)

IR (KBr) cm^{-1}: 3043.78 (Aromatic =C-H), 1595.02 and 1481.23 (Aromatic C=C), 692.40 (Aromatic C=C-H bending), 1394.44 (Alkane CH_3), 1654.18 (Imine C=N), 3340.48 (N-H stretching), 1552.59 (N-H bending), 1004.84 (-C-N), 1078.13 (Aromatic-Br). MS (ESI): 289.1 (M^+).

3-(4-bromophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (Ve)

IR (KBr) cm^{-1}: 3042.47 (Aromatic C-H), 1598.88 and 1478.39 (Aromatic C=C), 686.61 (Aromatic C=C-H bending), 1647.31 (C=C), 1598.88 and 1647.31 (C=C-CHO), 1091.63 (Aromatic-Br). MS (ESI): 327.1 (M^+). ^{1}H-NMR (δ/$CDCl_3$): δ 7.762-7.614 (t, 1H), δ 8.122-8.035 (t, 2H), δ 8.554-8.518 (d, 2H), δ 8.879-8.799 (d, 4H), δ 9.108 (s, 1H), δ 9.551 (s, 1H).

1-(1-(4-hydroxyphenyl)ethylidene)-2-phenylhydrazine (IIIf)

IR (KBr) cm^{-1}: 3018.39 (Aromatic =C-H), 1601.12 and 1487.01 (Aromatic C=C), 899.27 (Aromatic C=C-H bending), 1373.22 (Alkane CH_3), 1647.33 (Imine C=N), 3352.05 (N-H stretching), 1487.01 (N-H bending), 1373.22 (-C-N), 1026.08 (Aromatic-OH). MS (ESI): 226.6 (M^+).

3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (Vf)

IR (KBr) cm^{-1}: 3060.82 (Aromatic C-H), 1598.14 and 1453.81 (Aromatic C=C), 686.61 (Aromatic C=C-H bending), 1672.17 and 1641.61 (C=C-CHO), 3606.38 (Aromatic-OH). MS (ESI): 264.5 (M^+). ^{1}H-NMR (δ/$CDCl_3$): δ 6.954-6.904 (d, 2H), δ 7.232-7.187 (t, 1H), δ 7.603-7.514 (d, 2H), δ 7.823-7.899 (t, 2H), δ 8.117-8.091 (d, 2H), δ 8.468 (s, 1H), δ 9.064 (s, 1H), δ 9.490 (s, 1H).

1-(1-(4-chlorophenyl)ethylidene)-2-(2,4-dinitrophenyl)hydrazine (IIIg)

IR (KBr) cm^{-1}: 3066.69 (Aromatic =C-H), 1581.52 and 1469.66 (Aromatic C=C), 698.18 (Aromatic C=C-H bending), 1388.65 (Alkane CH_3), 1672.17 (Imine C=N), 3446.56 (N-H stretching), 1509.21 (N-H bending), 1342.29 (-C-N), 1080.16 (Aromatic-Cl), 1552.50 and 1342.29 (Aromaric-NO_2). MS (ESI): 335.0 (M^+).

3-(4-chlorophenyl)-1-(2,4-dinitrophenyl)-1H-pyrazole-4-carbaldehyde (Vg)

IR (KBr) cm^{-1}: 3042.81 (Aromatic C-H), 1598.88 and 1478.37 (Aromatic C=C), 899.81 (Aromatic C=C-H bending), 1701.21 and 1624.47 (C=C-CHO), 956.63 (Aromatic-Cl), 1525.59 and 1367.44 (Aromaric-NO_2). MS (ESI): 373.3 (M^+). ^{1}H-NMR (δ/$CDCl_3$): δ 7.623-7.589 (d, 2H), δ 7.815 (s, 1H), δ 8.151-8.089 (d, 2H), δ 8.498-8.413 (d, 1H), δ 8.792-8.709 (d, 1H), δ 9.117 (s, 1H), δ 9.491(s, 1H).

1-(1-(3,4-dichlorophenyl)ethylidene)-2-(2,4-dinitrophenyl)hydrazine (IIIh)

IR (KBr) cm^{-1}: 3081.72 (Aromatic =C-H), 1600.81 and 1490.87 (Aromatic C=C), 779.19 (Aromatic C=C-H bending), 1350.08 (Alkane CH_3), 1672.89 (Imine C=N), 3357.84 (N-H stretching), 1490.87 (N-H bending), 1350.08 (-C-N), 1072.89 (Aromatic-Cl), 1521.73 and 1350.08 (Aromatic-NO_2). MS (ESI): 369.4 (M^+).

3-(3,4-dichlorophenyl)-1-(2,4-dinitrophenyl)-1H-pyrazole-4-carbaldehyde (Vh)

IR (KBr) cm^{-1}: 3016.75 (Aromatic C-H), 1579.32 and 1478.34 (Aromatic C=C), 559.32 (Aromatic C=C-H bending), 1757.97 and 1674.10 (C=C-CHO), 1029.95 (Aromatic-Cl), 1532.67 and 1377.08 (Aromatic-NO_2). MS (ESI): 407.7 (M^+). ^{1}H-NMR (δ/$CDCl_3$): δ 7.490-7.452 (d, 1H), δ 7.632 (s, 1H), δ 7.919-7.893 (d, 1H), δ 8.119 (s, 1H), δ 8.498-8.471 (d, 1H), δ 8.801-8.754 (d, 1H), δ 9.025 (s, 1H), δ 9.423 (s, 1H).

1-(2,4-dinitrophenyl)-2-(1-(naphthalen-2-yl)ethylidene)hydrazine (IIIi)

IR (KBr) cm^{-1}: 3027.53 (Aromatic =C-H), 1604.35 and 1460.50 (Aromatic C=C), 669.25 (Aromatic C=C-H bending), 1381.44 (Alkane CH_3), 1705.31 (Imine C=N), 3252.20 (N-H stretching), 1510.39 (N-H bending), 1351.16 (-C-N), 1550.09 and 1381.44 (Aromatic-NO_2) MS (ESI): 351.0 (M^+).

Table 2: Physical characterization data of the compounds 3a-i and 5a-i

Compound Code	Colour	Solubility	Melting range ◦c	R_f value
(IIIa)	Yellow	$CHCl_3$	122-124	0.69
(IIIb)	Yellow	$CHCl_3$	110-113	0.59
(IIIc)	Yellow	$CHCl_3$	112-113	0.64
(IIId)	Yellow	$CHCl_3$	108-111	0.72
(IIIe)	Yellow	$CHCl_3$	126-128	0.65
(IIIf)	Yellow	$CHCl_3$	108-111	0.57
(IIIg)	White	$CHCl_3$	176-178	0.68
(IIIh)	White	$CHCl_3$	162-163	0.58
(IIIi)	White	$CHCl_3$	112-115	0.64
(Va)	Brown	$CHCl_3$	240-242	0.43
(Vb)	Brown	$CHCl_3$	220-222	0.38
(Vc)	Brown	$CHCl_3$	198-200	0.49
(Vd)	Brown	$CHCl_3$	222-225	0.59
(Ve)	Brown	$CHCl_3$	270-273	0.48
(Vf)	Brown	$CHCl_3$	254-257	0.37
(Vg)	Red	$CHCl_3$	162-165	0.45
(Vh)	Brown	DMSO	148-151	0.37
(Vi)	Orange	$CHCl_3$	138-140	0.41

1-(2,4-dinitrophenyl)-3-(naphthalen-2-yl)-1H-pyrazole-4-carbaldehyde (Vi)

IR (KBr) cm^{-1}: 3058.85 (Aromatic C-H), 1601.14 and 1471.15 (Aromatic C=C), 869.84 (Aromatic C=C-H bending), 1702.68 and 1654.04 (C=C-CHO), 1545.86 and 1367.44 (Aromatic-NO_2). MS (ESI): 388.9 (M^+). ^{1}H-NMR (δ/$CDCl_3$): δ 7.781-7.189 (t, 2H), δ 7.425 (s, 1H), δ 7.602-7.571 (d, 2H), δ 7.901-7.815 (d, 2H), δ 8.185-8.124 (d, 1H), δ 8.498 (s, 1H), δ 8.795-8.716 (d, 1H), δ 9.118 (s, 1H), δ 9.487 (s, 1H).

EVALUATION OF BIOLOGICAL ACTIVITY

Principle of DPPH (1,1-diphenyl-2-picrylhydrazyl) assay

DPPH assay is extensively used to evaluate antioxidant activities and is faster than with other methods. DPPH assay evaluates the capacity of compound to transfer a hydrogen atom (protection against lipid peroxidation and glutathione oxidation) and evaluate the capacity of compounds to transfer a single electron. DPPH is a stable free radical containing an odd electron in its structure and accepts an electron or hydrogen radical to become stable diamagnetic molecule. Usually utilizes for detection of the radical scavenging activity in chemical analysis. DPPH in ethanol shows a strong absorption band at 517 nm (independent of pH from 5.0 to 6.5), and the solution appears to be deep violet in colour. As

the DPPH radical is scavenged by the donated hydrogen from the antioxidant, the absorbance is diminished according to the stoichiometry and a solution converts to yellow in colour from deep violet. Although DPPH is a comparatively stable free radical at room temperature, it is not water soluble and the reaction mechanism between the antioxidant and DPPH radical depends on the structural conformation of the antioxidant [12].

DPPH assay procedure

Different concentrations (25, 50, 75, 100 µg/mL) of test and standard (ascorbic acid) compounds were prepared in methanol solution and added (3.0 mL) to the DPPH solution (1.0 mL, 0.1 mM) and allowed to stand for 30 minutes in dark. The free radical scavenging activity was determined by measuring the decrease in absorption at 517 nm in a UV-visible spectrophotometer. The actual decrease in absorption was measured against that of the control (0.2 mM, DPPH solution) [13].

Statistical analysis

The absorbance of the final reaction mixture of three parallel experiments was taken and is expressed as mean ± standard deviation. The activities were also determined as a function of their % inhibition which was calculated using the formula;

% Inhibition = (Ac – As / Ac) x 100

Where, Ac = absorbance of control, as = absorbance of sample.

The % inhibition of the tested compounds increased with the concentration represented increased radical scavenging activity. The line diagram for % inhibition versus concentration is given in fig. 1. IC_{50} number was calculated from equation of line obtained by plotting a graph of concentration versus % inhibition (Table 3). Bar diagram for IC_{50} values of synthesized compounds and standard ascorbic acid is given in fig. 2.

Principle of FRAP (ferric reducing antioxidant power) assay

FRAP assay measures the reducing power of the antioxidant molecule i.e. the FRAP assay also evaluate the capacity of compound to transfer a hydrogen atom (protection against lipid peroxidation and glutathione oxidation) and evaluate the capacity of compounds to transfer a single electron. Substances which have reduction potential reacts with potassium ferricyanide (Fe^{+++}) to form potassium ferrocyanide (Fe^{++}) which then reacts with ferric chloride ($FeCl_3$) to form ferric ferrous

complex that has an absorption maximum at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Ferric Ion Reducing/antioxidant Power (FRAP) assay procedure

Antioxidant activity was determined by FRAP assay as described by Oyaizu, 1986. According to this method four concentrations (25, 50, 75, 100 µg/mL) of each sample and standard in DMSO were prepared and mixed (2.5 mL) with phosphate buffer (2.5 mL, 0.2 mole, pH 6.6) and 1.0 % potassium ferricyanide (2.5 mL). The mixture was incubated at 50 ºC for 20 minutes.

Aliquots of 10 % trichloro acetic acid (2.5 mL) were added to the mixture, centrifuged at 5000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared ferric chloride solution (0.5 mL, 0.1 %) and allowed to stand for 30 minutes in dark to complete the reaction. The control solution was prepared as above, taking water in place of samples. The absorbance was measured at 700 nm.

Aliquots of 10 % trichloro acetic acid (2.5 mL) were added to the mixture, centrifuged at 5000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared ferric chloride solution (0.5 mL, 0.1 %) and allowed to stand for 30 minutes in dark to complete the reaction. The control solution was prepared as above, taking water in place of samples. The absorbance was measured at 700 nm.

***Preparation of Phosphate buffer (0.2 M, pH 6.6)*:** 18.75 mL of 0.2M dibasic sodium phosphate ($Na_2HPO_4.2H_2O$) was mixed with 31.25 mL of 0.2M monobasic sodium phosphate ($NaH_2PO_4.2H_2O$) and diluted to 100 mL with distilled water.

Statistical analysis

All tests and analysis were run in triplicates and the result obtained was averaged and expressed as mean ± standard deviation, linear relation (R^2) for both standard as well as samples was also calculated (Table 4). The absorbance of samples obtained at 100 µg/ml was converted to ascorbic acid equivalents using ascorbic acid standard curve (Y = mX + c) (Table 5).

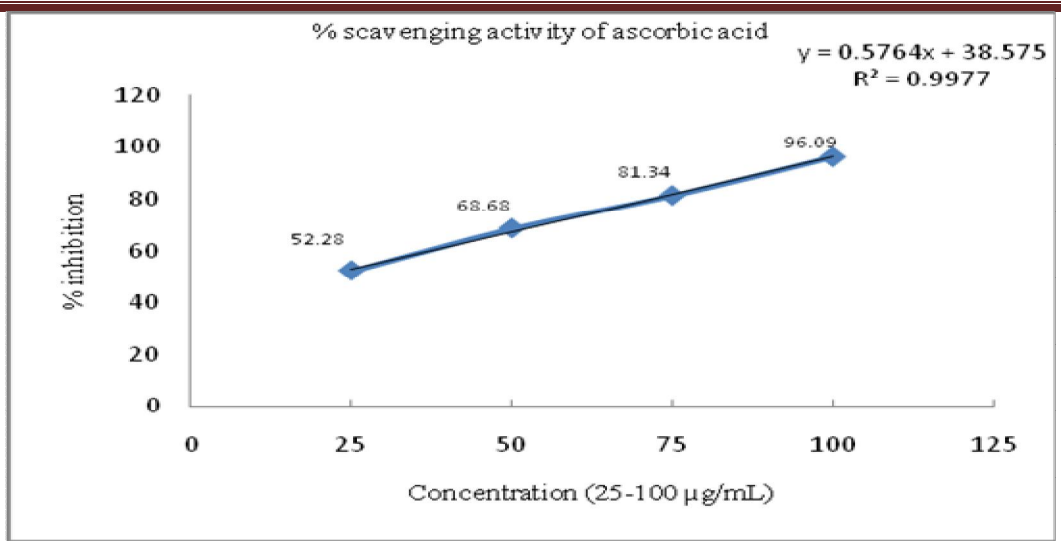


Fig. 1. Percent radical scavenging activity of standard antioxidant ascorbic acid

Table 3: DPPH radical scavenging activity of samples Va-i and standard antioxidant ascorbic acid

S.No.	Compound code	Concentration (µg/mL)	Absorbance at 517 nm	% Radical scavenging activity	IC_{50}
1	**V(a)**	25	0.556±0.004	43.55	80.83
2		50	0.525±0.004	46.70	
3		75	0.500±0.005	49.23	
4		100	0.471±0.008	52.18	
5	**V(b)**	25	0.534±0.003	45.78	70.41
6		50	0.515±0.009	47.72	
7		75	0.485±0.017	50.76	
8		100	0.465±0.012	52.79	
9	**V(c)**	25	0.524±0.008	46.80	56.50
10		50	0.501±0.006	49.14	
11		75	0.472±0.016	52.08	
12		100	0.446±0.003	54.72	
13	**V(d)**	25	0.551±0.018	44.06	75.75
14		50	0.519±0.009	47.31	
15		75	0.499±0.011	49.34	
16		100	0.462±0.008	53.09	
17	**V(e)**	25	0.539±0.008	45.27	71.40
18		50	0.519±0.002	47.31	
19		75	0.491±0.012	50.15	
20		100	0.459±0.011	53.40	
21	**V(f)**	25	0.509±0.001	48.32	38.82
22		50	0.478±0.001	51.47	
23		75	0.449±0.004	54.41	

24		100	0.414±0.007	57.96	
25	**V(g)**	25	0.574±0.006	41.72	93.99
26		50	0.529±0.001	46.29	
27		75	0.524±0.004	46.80	
28		100	0.483±0.012	50.96	
29	**V(h)**	25	0.573±0.007	41.83	90.21
30		50	0.528±0.004	46.40	
31		75	0.519±0.006	47.31	
32		100	0.479±0.003	51.37	
33	**V(i)**	25	0.576±0.019	41.52	97.32
34		50	0.530±0.007	46.19	
35		75	0.529±0.007	46.29	
36		100	0.486±0.003	50.66	
37	**Std.**	25	0.470±0.008	52.28	19.84
38		50	0.309±0.004	68.68	
39		75	0.184±0.001	81.34	
40		100	0.039±0.002	96.09	

Values are expressed as mean ± standard deviation (n = 3), absorbance of control 0.985 ± 0.002.

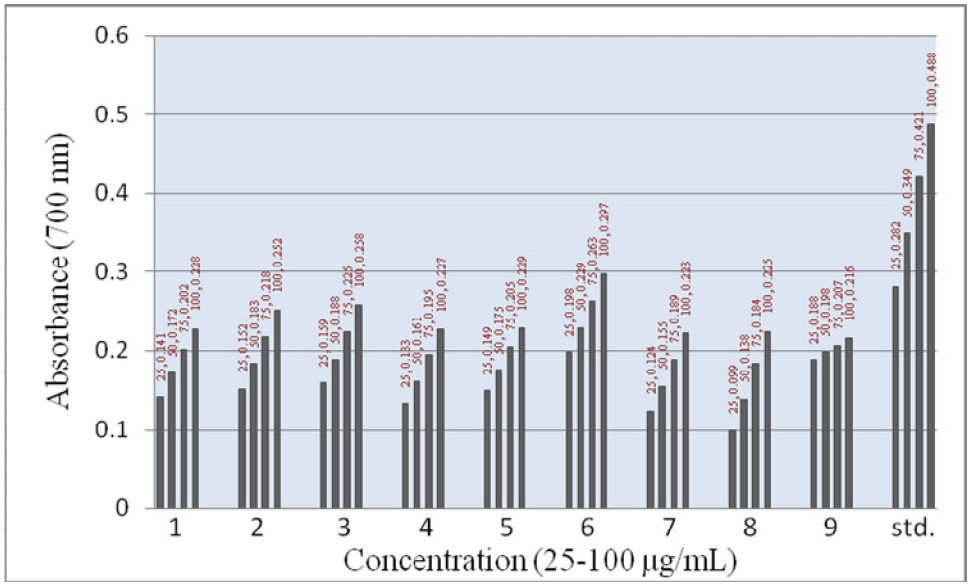


Fig. 3 Bar diagram for reducing power activity

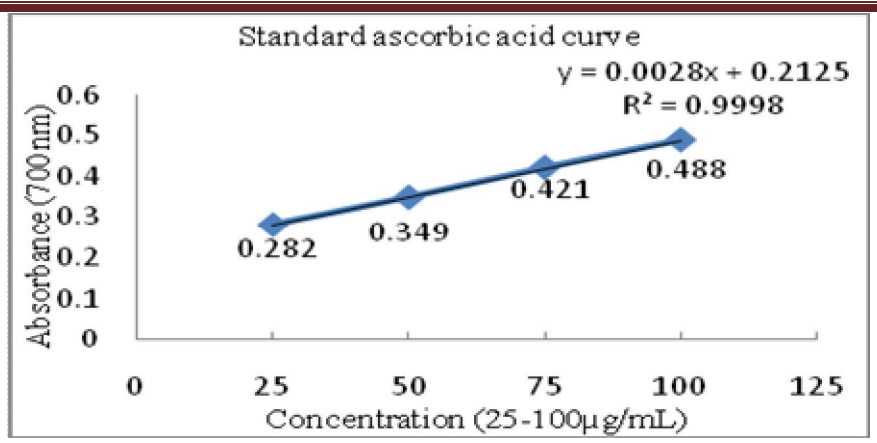


Fig. 4 Standard linearity curve of ascorbic acid

Table 4: FRAP activity of samples Va-i and standard antioxidant ascorbic acid

S. no.	Compound code	Concentration (µg/mL)	Absorbance at 700 nm	lienear relation (R^2)
1	**V(a)**	25	0.141±0.006	0.998
2		50	0.172±0.004	
3		75	0.202±0.007	
4		100	0.228±0.006	
5	**V(b)**	25	0.152±0.003	0.999
6		50	0.183±0.001	
7		75	0.218±0.007	
8		100	0.252±0.004	
9	**V(c)**	25	0.159±0.012	0.998
10		50	0.188±0.009	
11		75	0.225±0.004	
12		100	0.258±0.007	
13	**V(d)**	25	0.133±0.007	0.998
14		50	0.161±0.004	
15		75	0.195±0.004	
16		100	0.227±0.003	
17	**V(e)**	25	0.149±0.006	0.998
18		50	0.175±0.005	
19		75	0.205±0.003	
20		100	0.229±0.007	
21	**V(f)**	25	0.198±0.001	0.999
22		50	0.2299±0.003	
23		75	0.263±0.005	
24		100	0.297±0.001	
25	**VII(g)**	25	0.124±0.004	0.999
26		50	0.155±0.006	
27		75	0.189±0.004	
28		100	0.223±0.001	

29	**VII(h)**	25	0.099±0.004	0.997
30		50	0.138±0.006	
31		75	0.184±0.007	
32		100	0.225±0.003	
33	**VII(i)**	25	0.188±0.011	0.999
34		50	0.198±0.009	
35		75	0.207±0.013	
36		100	0.216±0.005	
37	**Std.**	25	0.292±0.002	0.999
38		50	0.359±0.004	
39		75	0.431±0.001	
40		100	0.498±0.002	

Values are expressed as mean±standard deviation (n = 3), absorbance of control 0.014±0.003.

Table 5: Concentration of samples (100 µg/mL) Equivalent to ascorbic acid (µg/mL)

S.No.	Test samples	Absorbance at 700 nm (100µg/mL)	concentration Equivalent to ascorbic acid (µgAAE/mL)
1	**V(a)**	0.228±0.006	8.00
2	**V(b)**	0.252±0.004	20.00
3	**V(c)**	0.258±0.007	23.00
4	**V(d)**	0.227±0.003	7.50
5	**V(e)**	0.229±0.007	8.50
6	**V(f)**	0.297±0.001	42.05
7	**VII(g)**	0.223±0.001	5.5
8	**VII(h)**	0.225±0.003	6.50
9	**VII(i)**	0.216±0.005	2.00

RESULTS AND DISCUSSION

All the synthesized compounds **(Va-i)** were screened for antioxidant activity by DPPH and FRAP assay. In DPPH assay the decline in absorbance was measured and the antioxidant capacity was determined. Among the synthesized derivatives, **Vf** has higher potency of radical scavenging, with good IC_{50}, which may be due to the presence of electron releasing hydroxyl group. According to the IC_{50}, antioxidant property of other compounds is in the order: Compound-**Vf** > Compound-**Vc** > Compound-**Vb** > Compound-**Ve** > Compound-**Vd** > Compound-**Va** > Compound-**Vh** > Compound-**Vg** > Compound-**Vi**. However, compounds VIIg, VIIh and VIIi showed lower potency with high values of IC_{50} (Table 3).

In FRAP assay, increased absorbance of the compounds with concentration indicates increased reducing power. Compounds with higher concentrations (100µg/mL) showed a higher reducing power (Fig. 3). The reducing power showed good linear relation (R^2) in both standard as well as compounds (Table 4). These results clearly reveal that compounds have antioxidant activity. Among the synthesized compounds, Vf showed maximum reducing activity which may be due to the presence of electron releasing hydroxyl group. The spectral data for the absorption at 100 µg/mL for the nine derivatives was compared with ascorbic acid. The plot was calculated by the equation: $y = 0.002x - 0.212$ ($R^2 = 0.999$) (Fig. 4) and it was found that the antioxidant property of the compounds in the order: Compound-**Vf** > Compound-**Vc** > Compound-**Vb** > Compound-**Ve** > Compound-**Va** > Compound-**Vd** > Compound-**Vf** > Compound-**Vg** > Compound-**Vh** > Compound-**Vi** (Table 5). It is expressed as AAE (ascorbic acid equivalent) which means that the reducing power of 100 µg/ml of each compound is equivalent to the reducing power of corresponding µg of ascorbic acid or expressed as µg AAE/mg of compound. Compound **Vc** is more potent than **Vb**, which may be due its good resonating structure due to the presence of two chloro substitutions. **Vd** is less potent than **Vb** which may be due to the less electronegative bromo substitution, providing weak resonating structure than **Vb** and **Vc**. **Va** is less potent than **Vb**, **Vc** and **Vd** which may be due to the unsubstituted phenyl ring at 3rd position of pyrazole-4-carboxaldehyde. **Vg**, VIIh and **Vi** showed lesser reducing power, which may be due to the presence electron withdrawing nitro groups on phenyl ring at 1st and 3rd position of pyrazole-4-carboxaldehyde [14, 15, 16, 17] .

CONCLUSION

We concluded that synthesized pyrazole-4-carboxaldehyde derivatives have antioxidant properties which may be due to the presence of a conjugated π-system, which delocalize after donation of hydrogen atom and stabilize the antioxidant molecule. Introduction of phenyl ring at first & third position of pyrazole may increase the antioxidant activity. The participation of the C=C bond is important in stabilizing the antioxidant radical by resonance. Introduction of electron releasing groups on phenyl rings attached to heterocycles increase the electron donating capacity of antioxidants. The presence of α,β-unsaturated ketonic functions in the pyrazole moiety may play an important role to act as a better electron donor which may enhance reducing power ability. Substitution of halogens to the phenyl ring may help for stabilization of the free radical form after electron donation and thus leading to enhanced reducing ability.

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