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Synthesis, characterization and pharmacological evaluation of substituted N-benzyl isatin 3-oximes

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ABSTRACT

"Isatin" is an Indole derivative identified in human beings considered as metabolic derivative of adrenaline used for the synthesis of Indoles, Quinolines, and as raw material for the synthesis of drugs. In this research a series of substituted N-Benzyl Isatin 3-oximes were synthesized by eletrophillic aromatic substitution and Beckmann rearrangement reactions and nucleophillic substitution reactions and characterized by IR, H^1NMR and Mass spectroscopy. Anti oxidant, Anthelmentic and Anti diabetic activities were evaluated for the compounds. The results shown that anong all the compounds chlorine substituted derivatives are most favorable compounds towards the activities.

INTRODUCTION:

Indole is an aromatic heterocyclic organic compound. The name Indole was obtained from the **Indigo** and **oleum** since Indole was first isolated by treatment of the indigo dye with oleum. It has a bicyclic structure, consisting of a six -membered benzene and pyrrole rings are fused through the 2- and 3-positions of the pyrrole nucleus. The participation of the nitrogen lone pair electron in the aromatic ring means that Indole is not a base, and it does not behave like a simple amine. The Indole ring is also found in many natural products such as the Indole alkaloids, fungal metabolites and marine natural products [(1)]

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It is a solid at room temperature and produced by bacteria as a degradation product of the amino acid tryptophan. It occurs naturally in human faces and it is a constituent of many flower scents (such as orange blossoms) and perfumes. It also occurs in coal tar.

Advantages:

1. Indole nucleus consists of plant hormone **Auxin** (indolyl-3-acetic acid), the anti-inflammatory drug indomethacin, the beta-blocker pindolol, and the naturally occurring hallucinogen dimethyl tryptamine.
2. Indigo can be converted to Isatin and then to oxindole. Then, in 1866, Adolf von Baeyer reduced oxindole to Indole using zinc dust[(2)] In 1869, he proposed a formula for Indole[(3)]
3. Indole derivatives used as dye stuffs.
4. It can also be present alkaloids like tryptophan and auxins.
5. It is having a wide variety of pharmacological activities like Analgesic, Anti allergic, Anticonvulsant, Antihistaminic, Anti-inflammatory, Antitumor etc.

Isatin:

Isatin(1H-indoline-2, 3-dione) is an endogeneous versatile substrate found in the mammalian brain , peripheral tissues, body fluids. The compound was first obtained by Erdman [4] and Laurent [5] in 1841 as a product from the oxidation of indigo dye by nitric acid and chromic acids. Isatin forms a blue dye if it is mixed with sulfuric acid and crude benzene. In nature, isatin is found in plants of the genus *Isatis* [6], in *Calanthe discolor* has also been found as a component of the secretion from the parotid gland of *Bufo* frogs and in humans as it is a metabolic derivative of adrenaline [7]

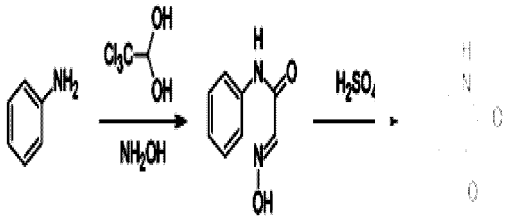
Physicochemical properties:

- Molecular formula: C8H5NO 2.
- Molar mass: 147.1308 g/mol.
- Appearance: Orange-red solid.
- Melting point: 200 °C, 473 K, 392 °F .

Synthesis of isatin:

The Sandmeyer methodology :

Isatin may be prepared from cyclicizing the condensation product of chloral hydrate, aniline and hydroxylamine in sulfuric acid. This reaction is called the Sandmeyer isonitrosoacetanilide . Isatin Synthesis and was discovered by Traugott Sandmeyer in 1919. The method applies well to Anilines with electron-withdrawing substituents, such as 2-Fluoroaniline.



Advantages:

- The reagents are cheap and readily available, and the yields are usually high.
- Sandmeyer methodology has been modified by the incorporation of ethanol as a co solvent.(8)
- Application of the modified Sandmeyer methodology allowed the synthesis of 4,6-dibromoisatin, a key intermediate for the synthesis of the marine natural product convolutamydine

Disadvantages:

- The use of N- alkyl anilines furnishes the corresponding N –alkyl isatins in low yield for example N-methyl isatin is obtained in 22% yield.

Materials and methods:

The chemicals and solvents used in this study were supplied by Merck (Darmstadt, Germany), Aldrich Chemicals Co. (Steinem, Germany) and SD Fine Chemicals, Mumbai. Melting points were determined in open capillaries on a Heco melting point apparatus. The purity of the compound was determined by Thin Layer Chromatography (TLC). The chemical structures were confirmed by elemental and spectral analysis. IR spectra were recorded on a SHIMADZU as KBr disc (γ, cm^{-1}). ^{1}H-NMR spectra were obtained on a BRUKER AC 399.65 MHz Spectrophotometer using TMS as an internal standard (Chemical shift in δ, ppm). Mass spectra were recorded on electron impact spectrophotometer at 70Ev using direct insertion probe.

General Procedure for the Synthesis of 5 chloro Isatin:

0.01mol of Isatin was added to the 0.001mol of TCCA containing 6ml of H_2SO_4 in an ice bath, after the complete addition of the Isatin, the ice bath was removed, and the mixture was kept stirring for 1 hr. The completion of reaction was monitored by using the solvent system ethyl acetate: hexane(1:1).The mixture was then poured over cracked ice. The precipitate was filtered under vacuum and washed with water. And solubulized in ethyl acetate, filtered and the solution was evaporated at reduced pressure to separate the isocyanuric acid formed as byproduct(9)

General Procedure for the synthesis of 5, 7 di chloro Isatin:

0.01mol of Isatin was added to the 0.006mol of TCCA(Tri chloro iso cyanuric acid) containing 6ml of H_2SO_4 in an ice bath, after the complete addition of the Isatin, the ice bath was removed, and the mixture was kept stirring for 1 hr. The complete of reaction was monitored by using the solvent system ethylacetate : hexane(1:1) and poured over cracked ice. The precipitate was filtered and washed with water. Subsequently, the product was solubulized in ethyl acetate, filtered and the solution was evaporated to separate the isocyanuric acid formed as byproduct. (10)

General Procedure for the synthesis of 5-bromo Isatin:

Isatin (0.01mol) was warmed in ethanol (95%, 20ml) with stirring until it was dissolved. Bromine (0.01mol) was added drop wise to the stirred Isatin solution by maintaining the temperature in between 70 to 75°C. The solution was cooled to room temperature and placed on ice bath for 30 min. The precipitate was washed with water and cold ethanol Then crystallized from ethanol to yield 5-bromoisatin. The reaction was monitored by TLC using solvent system Benzene: Ethanol (1:0.5) (11)

General Procedure for the synthesis of 5,7 dibromo Isatin:

Isatin (0.01mol) was warmed in ethanol (95%20mL) with stirring until it was dissolved. Bromine (0.04mol) was added drop wise to the stirred Isatin solution whilst maintaining the temperature of the reaction mixture between 70 to 75°C. The solution was cooled to room temperature and placed on ice bath for 30 min. The resulting precipitate was washed with water and cold ethanol .Then crystallized from ethanol to yield 5-bromoisatin. TLC was performed by using solvent system Benzene : Ethanol (1:0.5)[12]

General Procedure for the synthesis of 5-Nitro Isatin: [13]

Isatin (0.01mol) was dissolved in 4ml of acetic acid solution and then 1.8ml of sulphuric acid was added drop wise in the ice cold condition. Then nitric acid 0.6ml was added drop wise for sufficient time with stirring. The solution was placed in freezer for 24 hrs to get the solid product and then dried. It was monitored by TLC Using dichloro methane: methanol (1:0.5)

General Procedure for the Synthesis of substituted Isatin 3-oximes:

To a mixture of 0.01mol of substituted Isatin ,0.01mol of hydroxylamine hydrochloride,4ml of 95% of ethanol and 0.8ml of water. 0.05mol of powdered sodium hydroxide was added in small portions. The mixture was refluxed for 15mnts and poured into dilute HCl solution. The precipitated oxime obtained was directly pure. [14]

General Procedure for the Synthesis of substituted N-Benzyl Isatin 3-oximes:

To 0.01mol of substituted isatin3-oxime, anhydrous potassium carbonate (0.06mol), acetone (18.3 ml), were added and mixed together.

Then 0.006 mol of benzyl chloride was added drop wise. The mixture was stirred at room temp for about 8 hrs the reaction was monitored by TLC [ethyl acetate: hexane (1:1)] The mixture was then poured into water and extracted with ethyl acetate, dried over anhydrous sodium sulfate and concentrated under vaccum to give the pure compound. Desired compound was re crystallized from ethanol.[15]

Spectral data for the synthesized compounds: 1-benzyl-3(hydroxyimino)indolin 2-one(BIO-1) :

IR (cm^{-1},KBr) : CH(S) in Ar-3012.81cm^{-1},C=Cstr-1548 cm^{-1},enolic OH (S) 3000 cm^{-1},CH str in CH_2 -2845 cm^{-1},CH def in CH_2-1456 cm^{-1},C=Ostr-1739 cm^{-1},C-N str in benzyl-1456 cm^{-1},C-N=O str in oximes-1653,1386 cm^{-1}. NMR (399.65MH_Z,CDCl3,δ ppm) : 2.4(s,1H=N-OH), 7.3 (m,1H-H4 of Isatin) , 6.9 (d,1H,H-5 of Isatin J=0.799H_Z), 6.7 (d H-6 of Isatin), 4.4 (d H_2 of benzyl) 7.37(t , Ar-H J=3.99H_Z)

1-benzyl-5-chloro-3-(hydroxyimino) indolin-2-one (BIO-2):

IR(cm^{-1},KBr): C-H(S) in Ar-2964.59cm^{-1},C=Cstr-1552.7 cm^{-1}, enolic OH (S)-2908cm^{-1}, CH str in CH_2 -2843cm^{-1}, CH def in CH_2-1446 cm^{-1}, C=Ostr-1745.58 cm^{-1},C-Nstr in benzyl-1463 cm^{-1}, C=N-OH str in oximes-1660.71(S), Mono substituted C-H(def)-693.3(S) cm^{-1}, C-Cl-693.3(S) cm^{-1}

1-benzyl-5,7-dichloro-3-(hydroxyimino)indolin-2-one(BIO-3):

IR (cm-1,KBr) : C-H(S) in Ar-2964.59cm^{-1},C=Cstr-1552.7 cm^{-1}, enolic OH (S)-2908cm^{-1}, CH str in CH_2 -2843cm^{-1}, CH def in CH_2-1446 cm^{-1}, C=Ostr-1745.58 cm^{-1},C-Nstr in benzyl-1463 cm^{-1}, C-N=O str in oximes-1660.71(S), 1377.17(S) cm^{-1}, Di substituted ortho & para C-H(def)-493.78(S) cm^{-1}, C-Cl-693.3(S) cm^{-1}. NMR (DMSO-d_6-δ ppm): 1.9 (1H=N-OH) ,7.3(d,1H,H4 of Isatin J=1.598H_Z) 7.9 (1H, H-6 of Isatin) ,5.4(t,H_2 of benzyl)

MASS : 321.16 (M+2, M+4=320.1)

1-benzyl-5-bromo-3-(hydroxyimino) indolin-2-one (BIO-4):

IR(cm-1,KBr): CH(S) in Ar-3095.75cm^{-1},C=Cstr-1550.7 cm^{-1},enolic OH (S)-2914.44cm^{-1}, CH str in CH_2 -2852.72cm^{-1}, CH def in CH_2-1456.26 cm^{-1}, C=Ostr-1734.01 cm^{-1},C-Nstr in benzyl-1496.76 cm^{-1}, C-N=O str in oximes-1680.(S), 1394.53(S) cm^{-1}, Mono substituted C-H(def)-694.37(S) cm^{-1}, C-Br-694.37 (S) cm^{-1}.

1-benzyl-5, 7 dibromo-3-(hydroxyimino) indolin-2-one (BIO-5)

IR (cm-1, KBr):

CH(S) in Ar-3425.51cm^{-1},C=Cstr-1496.76 cm^{-1}, OH (S)-2725,42cm^{-1}, CH str in CH_2 -2374.37cm^{-1}, CH def in CH_2-1460.11cm^{-1}, C=Ostr-1734.01 cm^{-1},C-Nstr in benzyl-1496.76 cm^{-1}, C-N=O str in oximes-1653.(S), 1375.25(S) cm^{-1}, Di substituted ortho & para C-H(def)-721 (S) cm^{-1},889.18 C-Br-530.01 (S) cm^{-1}.

NMR (DMSO-d_6-δ ppm): 1.8 (**s,** 1H=N-OH), 7.4(d,1H, H-4 of Isatin,J=6.794),7.9 (M 1H, H5,H-6 of Isatin),5.4 (s, H_2 of benzyl).

MASS: 410 (408.8) (M+2)(M+4)

1 -benzyl-3-(hydroxyimino) 5-nitroindolin-2-one (BIO-6):

IR(cm-1,KBr): CH(S) in Ar-3425.51cm^{-1},C=Cstr-1496.76 cm^{-1}, OH (S)-2725,42cm^{-1}, CH str in CH_2 -2374.37cm^{-1}, CH def in CH_2-1460.11cm^{-1}, C=Ostr-1734.01 cm^{-1},C-Nstr in benzyl-1496.76 cm^{-1}, C-N=O str in oximes-1653.(S), 1375.25(S) cm^{-1}, Di substituted ortho & para C-H(def)-721 (S) cm^{-1},889.18 C-Br-530.01 (S) cm^{-1}.

Free radical scavenging assay (DPPH)

For the evaluation of antioxidant activity, a stable free radical, α,α-diphenyl-β-picryl hydrazyl (DPPH) was used at the concentration of 0.004% in methanol[16]. To the 2 ml of test compound (5, 10,15,20,25 µg/ml), 2 ml of DPPH solution were added, mixed thoroughly the reaction mixture was incubated in a dark place for 30 min. In addition, absorbance (OD) was measured at 517 nm against

blank. The % reduction of free radical concentration (OD) with different concentration of test compounds was calculated and compared with standard, ascorbic acid. The results were expressed as IC_{50}. Percentage inhibition of DPPH radical scavenged was calculated according to the formula:

Percentage scavenged = $\mathbf{((A_c - A_s)/A_c) * 100}$

A_C = absorbance of control

A_S = absorbance of test

***In vitro* anti diabetic activity:**

Starch-Iodine colour assay:

Screening of synthesized compounds for α-amylase inhibitors was carried out according to Xiao et al. (2006). various concentrations (5,10,15,20,25 µg/ml), of drug solutions (test compounds like BIO-1-6 and standard drug acarbose) in 500 µL were added to 500 µL of 0.02 M sodium phosphate buffer (P^H -6.9 containing 6mM sodium chloride) containing 0.04 units of α-amylase solution and were incubated at 37°C for 10 min. Then 500 µL soluble starch (1%, w/v) was added to each concentration of test compounds and incubated at 37°C for 15 min. 1 M HCl (20 µL) was added to stop the enzymatic reaction, followed by the addition of 100 µL of iodine reagent containing 635mg Iodine and 1gm potassium iodide in 250ml distilled water. The colour change was noted and the absorbance was readed at 620 nm. [17] Inhibition of enzyme activity was calculated as:

Inhibition of enzyme activity (%) = (C-S) / C × 100,

Where S = absorbance of the sample

C = absorbance of blank (no test compounds).

Anti helminthic activity:

Anti helmintic activity for synthesized compounds was estimated by earth worms. Five earth worms of nearly equal size were placed in standard drug solution and test compound solutions at room temperature using Normal saline as control. The standard drug and test compounds were dissolved in minimum quantity of dimethyl formamide (DMF) and the volume was adjusted up to 15 mL with normal saline solution to get the concentration of 0.1% w/v, 0.2% w/v and 0.5% w/v. Albendazole was used as a standard drug. The compounds were evaluated for the time taken for complete paralysis and death of earth worms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time. To ascertain the death of motionless worms, external stimuli should be frequently applied ,which stimulate and induces movement in the worms, if alive. [18]

Discussion of results: The presence of various reaction centers on Isatin render them capable of participating in more no of reactions leading to its extensive use as a precursor molecule in medicinal chemistry. In this study various new compounds have been synthesized by using Isatin as a starting material. All the synthesized compounds were characterized by FTIR, NMR, MASS spectroscopic techniques.

Free radical scavenging activity: The synthesized Isatin derivatives (BIO 1-6) were subjected for free radical scavenging activity at different concentrations (5-25 µg/ml). Among all the compounds BIO-3 having electron with drawing group like chlorine at C-5 &C- 7 position exhibited highest activity than that of remaining compounds. The IC_{50} value of BIO-3 was found to be 11µg/ml. The IC_{50} value of standard was found to be 8µg/ml. The keto lactum ring is responsible for the free radical scavenging activity due to its N-H & C=O moieties, so all the compounds exhibited moderate free radical scavenging activity. [19]

***In vitro* anti diabetic activity:**

The synthesized compounds were tested for *in vitro* anti diabetic activity by starch iodine color assay method. Among all the synthesized compounds BIO-3 was more active, having Chlorine at C-5 and C-7 positions. The IC_{50} value of BIO-3 was found to be 10µg/ml.

***In vitro* anti helminthic activity:**

All the derivatives were screened for anti helminthic activity using *perithima posthuma.* The activity was compared with a standard drug albendazole. All the derivatives were tested at various concentrations like 0.1, 0.2, 0.5 % w/v. and their Paralytic and death time was noted. By increasing the concentration of the compounds the paralytic and death time was decreased. All the compounds showed less time for the paralysis and death of the earth worms than the standard (Albendazole). Experimental results suggested that introduction of electron withdrawing groups (Cl, Br, NO_2) greatly enhances the biological activity of the Isatin.

Conclusion: All the title compounds (BIO1-6) were synthesized and purified. The structures were confirmed by spectral results. The synthesized compounds were evaluated for biological activities like antioxidant, anti diabetic & anti helminthic activity. According to the experimental results it was concluded that the compounds having electron withdrawing groups like Cl, Br and NO_2 at C-5 & C-7 position enhances the biological activity than BIO-1, but among all the compounds chlorinated Isatin derivative substituted at 5 and 7 positions may greatly increases the biological activity.

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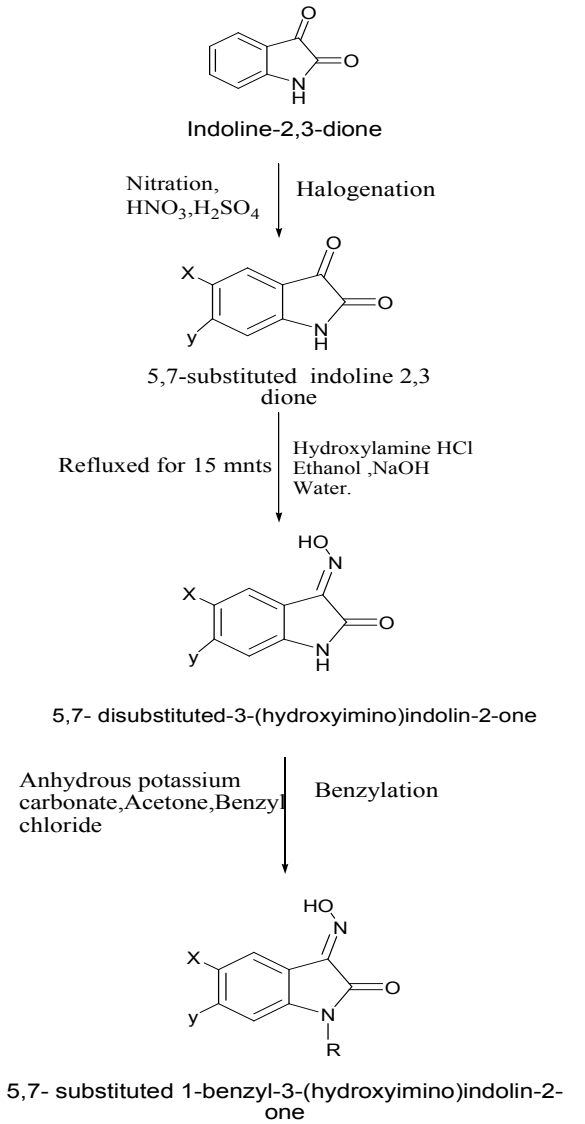

Compound no	Mol.Formula	x	y	Mol. Wt	M.P ^{0} C	R_f Value	Yield %
BIO-1	$C_{15}H_{12}N_2O_2$	H	H	252.2	220-222	0.4	70.5
BIO-2	$C_{15}H_{11}ClN_2O_2$	Cl	H	286.7	228-230	0.7	78
BIO-3	$C_{15}H_{10}Cl_2N_2O_2$	Cl	Cl	321.1	233-235	0.8	75
BIO-4	$C_{15}H_{11}BrN_2O_2$	Br	H	331.1	234-236	0.6	68
BIO-5	$C_{15}H_{10}Br_2N_2O_2$	Br	Br	410	240-242	0.7	65
BIO-6	$C_{15}H_{11}N_3O_4$	NO_2	H	297.2	210-212	0.8	66

Table no1: Physical data of the synthesized compounds:

Table no 2: Anti oxidant activity of synthesized compounds:

Con	BIO-1		BIO-2		BIO-3		BIO-4		BIO-5		BIO-6	
	Absorbance	% inh	Absorbance	% inh	Absorbance	% inh	Absorbance	% inh	Absorbance	% inh	Absorbance	% inh
5	0.561±0.006	35.1	0.527±0.003	39	0.517±0.004	40.2	0.533±0.002	38.3	0.524±0.002	39.4	0.576±0.001	33.4
10	0.518±0.004	40.1	0.468±0.002	45.8	0.453±0.003	47.6	0.481±0.003	44.3	0.472±0.002	45.4	0.492±0.002	43.1
15	0.473±0.005	45.3	0.389±0.002	55.1	0.349±0.002	59.6	0.408±0.003	52.8	0.394±0.001	54.4	0.446±0.002	48.4
20	0.413±0.002	52.2	0.259±0.001	70	0.229±0.004	73.5	0.281±0.001	67.5	0.276±0.003	68	0.391±0.002	54.7
25	0.324±0.002	62.5	0.202±0.002	76.6	0.195±0.001	77.5	0.226±0.001	73.8	0.218±0.001	74.7	0.258±0.001	69.3

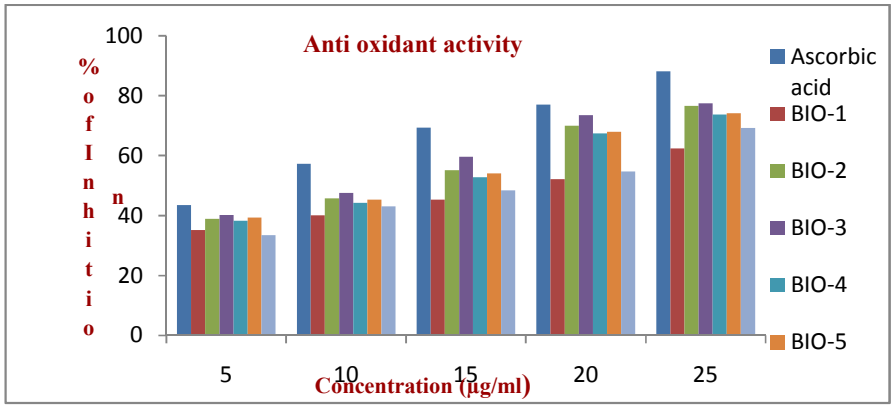

Table no 3: IC_{50} values of Anti oxidant activity of Standard and BIO 1-6 by DPPH method

S.no	Compound	ic_{50} values
1	STD	8
2	BIO-1	19
3	BIO-2	13
4	BIO-3	11
5	BIO-4	14
6	BIO-5	14
7	BIO-6	16

Table no 4: Anti oxidant activity of Ascorbic acid by DPPH method:

S.no	Concentration(µg/ml)	Absorbance	% of inhibition
1	5	0.488±0.004	43.5
2	10	0.369±0.003	57.3
3	15	0.264±0.001	69.4
4	20	0.198±0.001	77.1
5	25	0.052±0.004	88.2

Table no-5: Anti diabetic activity of standard by starch iodine color assay method

S.No	Concentration	Absorbance	% of inhibi-tion
1	5	0.495±0.003	46.6
2	10	0.382±0.007	58.7
3	15	0.256±0.007	72.3
4	20	0.236±0.007	74
5	25	0.126±0.005	86

Table no - 6: Anti diabetic activity of BIO 1-6 by starch iodine color assay method

Con	BIO-1		BIO-2		BIO-3		BIO-4		BIO-5		BIO-6	
	Abs	**%inh**	**Abs**	**%inh**	**Abs**	**%inh**	**Abs**	**%inh**	**Abs**	**%inh**	**Abs**	**%inh**
5	0.572±0.001	38.2	0.532±0.004	42.6	0.512±0.009	44.7	0.559±0.006	39.6	0.551±0.006	40.5	0.567±0.004	39.6
10	0.526±0.002	43.2	0.498±0.005	46.2	0.461±0.004	50.2	0.502±0.006	45.8	0.498±0.006	46.2	0.504±0.006	44.4
15	0.492±0.001	46.9	0.426±0.006	54	0.345±0.001	62.7	0.458±0.005	50.5	0.424±0.003	54.2	0.456±0.008	50.8
20	0.401±0.002	56.6	0.366±0.007	60.5	0.282±0.007	69.5	0.397±0.006	57.1	0.390±0.005	57.9	0.386±0.006	59.6
25	0.358±0.002	61.3	0.258±0.007	72.1	0.210±0.009	77.3	0.325±0.008	64.9	0.278±0.007	70	0.307±0.007	65.4

Table no - 7:IC_{50} values of anti diabetic activity of standard and BIO-1-6 by starch- iodine colour assay

S.No	COMPOUND	IC_{50} VALUES
1	STD	7
2	BIO-1	17
3	BIO-2	12
4	BIO-3	10
5	BIO-4	15
6	BIO-5	13
7	BIO-6	14

Table no-8: Anthelminthic acticity of standard and BIO-1-6

s.no	comp	conc. (% w/v)	for paralysis	for death
1	Control	0.9	-	-
2	Standard	0.1 0.2 0.5	32.42 ±1.744 32.17 ± 1.602 31±1.414	75.17 ± 1.169 73 ± 1.140 70.33 ± 1.211
3	BIO-1	0.1 0.2 0.5	24.17±1.412 24 ± 1.414 21.17 ± 1.472	67.50 ± 1.032 63.52 ± 0.970 56.36 ± 1.082
4	BIO-2	0.1 0.2 0.5	17 ± 1.414 14 ± 1.414 10.5 ± 1.049	55.23 ± 0.752 53 ± 0.707 43 ± 1.966
5	BIO-3	0.1 0.2 0.5	13.38 ± 1.169 11.33 ± 1.033 7.167 ± 1.329	43.42 ± 1.111 40.08 ± 1.489 33.53 ± 1.054
6	BIO-4	0.1 0.2 0.5	21 ± 1.414 19 ± 1.414 15.50 ± 1.871	58.07 ± 1.306 55.5 ± 1.517 48.77 ± 1.242
7	BIO-5	0.1 0.2 0.5	20.67 ± 3.266 15 ± 1.414 14.17 ± 2.483	54.5 ± 1.378 44.8 ± 1.191 35.6 ± 2.388
8	BIO-6	0.1 0.2 0.5	23 ± 1.414 22.5 ± 1.414 18.17 ± 2.229	65.48 ± 2.155 62 ± 2.098 54.22 ± 1.270

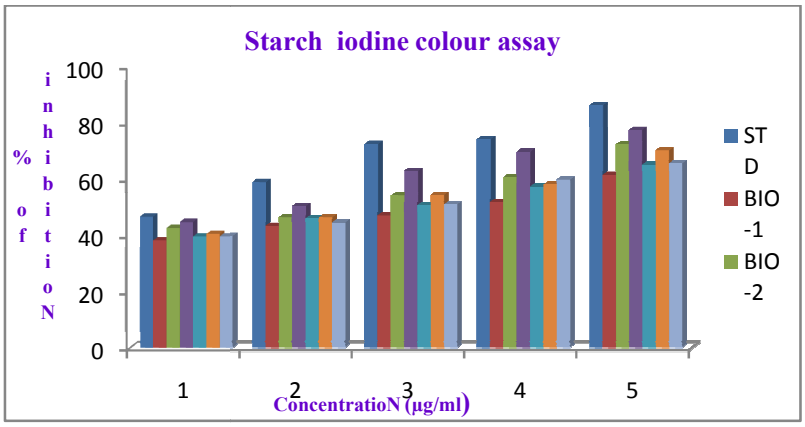

Fig 1: Graphical representation of standard and BIO-1-6 by Starch iodine colour assay method

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