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ISOLATION OF β-RESORCYLADEHYDE FROM *Cassia Fistula* BARK- A NOVEL METHOD, ITS CHARACTERIZATION, STUDY OF ITS EFFECT ON GLUCOAMYLASE, α-AMYLASE, *In-vitro,* AND ANTIOXIDANT PROPERTIES

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ABSTRACT

The current study focuses on the novel method of isolation of β-Resorcyladehyde from *Cassia Fistula* bark extracts. The compound was further purified from the ethyl acetate extract. The column chromatography technique with various compositions of eluents has been employed for purification. The purified compound was then characterised by various spectrophotometric techniques. The ethyl acetate extract of *Cassia Fistula* bark showed no significant inhibition activity against glucoamylase and α-amylase but showed moderate DPPH radical scavenging activity.

Keywords: Novel Method, B-Resorcyladehyde, Column Chromatography Technique, Glucoamylase and α-amylase Inhibition, DPPH Radical Scavenging Activity.

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INTRODUCTION

β-Resorcyladehyde is a natural product found in many plants. The IUPAC name of the compound is 2,4-dihydroxybenzaldehyde or 4-formylresorcinol. The efficient method for the synthesis of this compound is the Vilsmeier-Haack reaction using resorcinol in the presence of Dimethylformamide and phosphorus oxychloride.[1,] The reaction is as follows (Fig.-1).

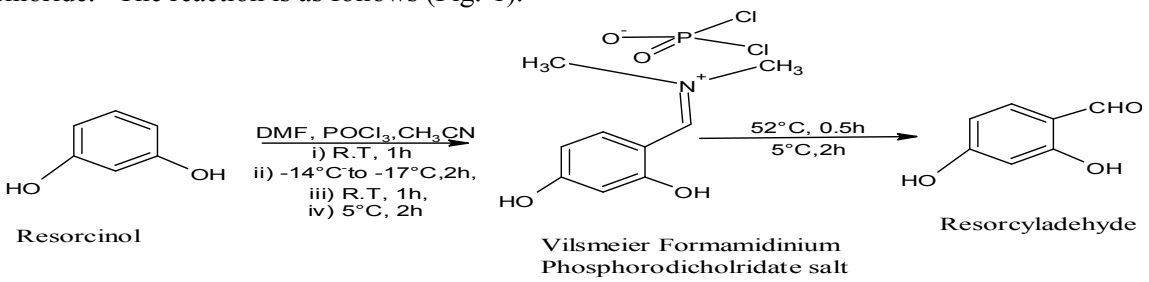


Fig.-1: Synthesis of β-Resorcyladehyde via Vilsmeier- Haack Reaction

The Schiff bases derived from β-Resorcyladehyde show potential biological activities. Transition metal complexes of β-Resorcyladehyde have been synthesised; the palladium complex showed the excellent antioxidant activity.[2] In another study, a novel ligand 2,4-dihydroxybenzaldehyde N(4)-methyl(phenyl)thiosemicarbazone has been synthesised and ligated with Co(II), Ni(II), and Cu(II). The cobalt and copper complexes show remarkable antifungal and antibacterial activity.[3] A series of silver(I) complexes of β-Resorcyladehyde-amino acid Schiff bases was synthesised, and their effects on α-glucosidase were investigated. The results show that all silver complexes show excellent inhibitory properties towards α-glucosidase and therefore can be suggested as an alternative therapeutic tool in the inhibition of α-glucosidase.[4] Two β-Resorcyladehyde Schiff bases, namely HIMB and HAMB, were synthesised and their effect on the corrosion of steel in 1 M HCl has been studied. It was observed that these could be able to suppress both anodic and cathodic reactions in an acidic electrolyte, thereby decreasing the corrosion of steel to a larger extent.[5] One group of researchers have synthesized amino acid

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methyl ester Schiff bases by condensation of amino acid methyl esters with β-Resorcyladehyde and further evaluated for the cytotoxic assay against A549, HeLa, HT29, Hep3B, MCF7, and C6 cells. It was investigated that β-Resorcyladehyde Schiff base gave positive results for all cell lines, but strong results were obtained for the C6 line as compared to other lines, making it an anti-cancer agent.[6] A Series of novel Schiff bases of β-Resorcyladehyde were prepared and tested for antifungal, antioxidant, antibacterial, and molecular docking studies. All compounds showed all activities in the moderate to good range.[7] out of 10 drugs designed could be used as safe oral drugs. Molecular docking studies showed the good binding ability of these compounds with DNA.[7] In one of the research β-Resorcyladehyde condensed with benzoylhydrazide gave a hydrazone Schiff base, which was subsequently used for the synthesis of various transition metal complexes such as V, Zn, Mo, Ru, and Pd. The study reveals that the Schiff bases behaved as a tridentate ligand. Amongst all complexes, the vanadium-ligated complex showed the highest antioxidant activity and high binding ability with DNA and caused significant DNA cleavage.[8] Novel polyurethane compounds derived from β-Resorcyladehyde were found to possess high thermal stability and hence could be used for aerospace industries.[9] This paper explores a novel method for the isolation of β-Resorcyladehyde from the bark of *Cassia Fistula* bark, the effect of the extract on glucoamylase and α-amylase and its antioxidant activity.

EXPERIMENITAL

Material and Methods

The chemicals used were of analytical grade, i.e., petroleum ether, Chloroform, Ethyl acetate, Ethanol, acetate buffer (pH 4.5), starch solution, DNS (3,5-dinitrosalicylic acid), Methanol, phosphate buffer solution (pH 7.0), 1,4-dioxane, DPPH (2,2-diphenyl-1-picrylhydrazyl), hexane, silica gel-H, silica gel (mesh 60-120) slurry. The IR spectra of the compounds were recorded on a Shimadzu IR Affinity FTIR spectrophotometer using KBr discs, and the values are expressed in cm-1. The 1H NMR spectra of compounds were recorded on a Bruker Avance II 400 MHz NMR spectrophotometer using DMSO as an internal standard, and the values are expressed in δ ppm. The elemental analyses of the compounds were recorded on an Elemental Vario El III, Carlo Erba 1108 Elemental analyzer. The structures of synthesised compounds were confirmed by spectral and elemental analysis.

General Procedure

Preparation of *Cassia fistula Bark* Extract and Its Phytochemical Study

The Bark of *Cassia fistula* was collected from the University Campus. They were air-dried for 6-7 days and ground to a coarse powder in a grinder. The powdered bark (100g) was soaked in methanol at room temperature for 7days, replacing the solvent after every 24 hrs. After the extraction, the solvent was removed by means of a rotary evaporation under vacuum, yielding the extract. The total extract (60g) was obtained. The methanol extract was further subjected to the liquid-liquid extraction using different immiscible solvents, usually water and an organic solvent. The procedure was repeated using solvents namely Petroleum ether (P.E), Chloroform ($CHCl_3$), and Ethyl Acetate (EtOAc). The obtained extracts were weighed and stored at +4°C in the refrigerator. These extracts were further analysed for phytochemical screening. To study the effect of extracts on glucoamylase in vitro, 0.5mL of the reaction mixture containing 0.1mL of extracts ranging from 20-100µg/mL, 0.3mL of 100mM acetate buffer (pH 4.5), and 0.1mL of glucoamylase (1.3 µg) was incubated at 37° C for 30 minutes. Then 0.5mL of starch solution (5mg/mL prepared in 100mM acetate buffer pH 4.5) was added and incubated further at 37° C for half an hour. The reaction was then terminated by placing the test tubes in a boiling water bath for 1-2 minutes, then cooled under running tap water. 2mL of DNS (3,5-dinitrosalicylic acid) reagent was added, and the test tubes were kept in a boiling water bath for 15 min. Then the test tubes were cooled and diluted with 7mL distilled water. The absorbance was recorded at 530 nm using a spectrophotometer, and the liberated glucose was estimated. A unit activity (U) is defined as the mg of glucose liberated per mg of protein per minute. 20-100µg/mL solutions of all the extracts were prepared in double-distilled water. 20-100µg/mL solutions of all the extracts were prepared in 1,4-dioxane, and the control for the same was also prepared. The results obtained were compared with acarbose, which is used as the positive control. To examine the effect of the extracts on α-amylase *in vitro,* 0.5mL of the reaction mixture

containing 0.1 mL of extract ranging from 20-100µg/mL, 0.3mL of 20mM phosphate buffer solution (pH 7.0) and 0.1mL of α-amylase (0.4 µg) was incubated at 37^0C for 30 minutes. Then 0.5mL of starch solution (5mg/mL prepared in 20mM phosphate buffer pH 7.0) was added and incubated further at 37° C for 30 minutes. The reaction was terminated by keeping the test tubes in a boiling water bath for 1-2 minutes, then cooled under running tap water. 2mL of DNS (3,5-dinitrosalicylic acid) reagent was added, and the test tubes were kept in a boiling water bath for 15 min. Then, the test tubes were cooled and diluted with 7mL distilled water. The absorbance was recorded at 530 nm using a spectrophotometer, and the liberated glucose was estimated. A unit activity (U) is defined as the mg of glucose liberated per mg of protein per minute. 20-100 µg/mL solutions of all the extracts were prepared in double-distilled water. 20-100µg/mL solutions of all the extracts were prepared in 1,4-Dioxane, and the control for the same was also prepared. The extracts were further analysed for their effect on DPPH radical scavenging activity 1mg/mL solutions of the extracts were prepared in Methanol. 0.2mM of the solution of DPPH in methanol was prepared, and 1.0mL of this solution was added to 1mL of the extract solution in methanol at different concentrations (100,200, 300, 400, 500µg/mL). After half an hour, the absorbance was measured at 517nm. A blank was prepared without adding the extract. Ascorbic acid and BHT were used as the standards. The ethyl acetate extract showed moderate DPPH radical scavenging activity. Therefore, we decided to purify the ethyl acetate extract.

Purification of Extract & Isolation of the Compound

Acidic Hydrolysis

10g of compound was refluxed with 10% H_2SO_4-MeOH (1:2, 15 ml), and then it was poured into ice water and again extracted with ethyl acetate. The ethyl acetate layer was washed with water and dried over $MgSO_4$ and concentrated to give the crude extract.

Column Chromatography

The ethyl acetate extract obtained after hydrolysis was subjected to conventional column chromatography. The column was packed with neutral silica gel of mesh size 60-120. The slurry was prepared by dissolving the compound in methanol. The system used for column chromatography is Hexane: Ethyl acetate system. In a 5:95 Hexane: Ethyl acetate system, a clear solution was obtained. This solution was concentrated and stored, which resulted in the appearance of colourless crystals. The crystals were washed with pet ether and later on treated with chloroform, which resulted in sharp needle-like crystals.

Detection Method

The compound obtained after purification was further subjected to structure elucidation by ^{1}H-NMR, ^{13}C-NMR, EI-MS, DEPT-135, IR and Elemental Analysis.

RESULTS AND DISCUSSION

Powdered *Cassia fistula bark* was subjected to extraction with various solvents in increasing order of polarity. The yield obtained from each solvent extract is shown in the Table-1.

Table-1: The Yield Obtained from Each Solvent Extract

S. No.	Extract	% yield
1	P.E	4.81
2	$CHCl_3$	7.27
3	EtOAc	12.46

All extracts obtained were further subjected for phytochemical screening to study the phyto-constituents present in the extracts. Data are shown in Table-2.

Table-2: Phytochemical Screening of Extracts

Phytochemical	P. E	$CHCl_3$	EtOAc
Alkaloids	-	+	+
Carbohydrates	-	-	+
Flavonoids	-	+	-
Triterpenoids	+	+	+

Saponins	-	+	-
Steroids	+	+	+

The results obtained for the Effect of the extracts on Glucoamylase *in vitro* were compared with acarbose, which is used as the positive control and tabulated as shown in Table-3.

Table-3: Effect of the Extracts on Glucoamylase, *in vitro*

Concentration (100µg/mL)	% Inhibition			
	P. E	$CHCl_3$	EtOAc	Acarbose
20	2.27	-2.27	4.82	23.85
40	2.83	0.85	5.67	34.77
60	6.23	4.82	9.35	43.67
80	18.41	12.18	13.03	55.17
100	25.50	20.40	22.95	58.90
IC_{50}	196.07	245.09	217.86	74.85

From Table-3, we observed that the glucoamylase inhibition is concentration-dependent. IC_{50} values of P.E, $CHCl_3$, and EtOAc are 196.07, 245.09, and 217.86 µg/mL, respectively. When we compared the IC_{50} value of Acarbose (74.85 µg/mL) with the IC_{50} values of all the extracts, it was observed that the P.E extract showed the lowest IC_{50} value. Further, the % inhibition of P.E, $CHCl_3$, and EtOAc at 100 µg/mL was 25.50, 20.40, and 22.95%, respectively. The results obtained for the Effect of the extracts on α-amylase *in vitro* were compared with acarbose, which is used as the positive control, and tabulated as shown in Table-4.

Table-4: Effect of the Extracts on α-amylase, *in vitro*

Concentration (100µg/mL)	% Inhibition			
	P.E	$CHCl_3$	EtOAc	Acarbose
20	-0.95	-6.07	2.23	21.72
40	8.94	-4.15	2.55	28.75
60	11.18	7.987	5.11	37.38
80	13.41	10.22	9.90	46.32
100	27.79	22.04	14.05	58.46
IC_{50}	179.92	226.80	355.66	85.18

From Table-4, we observed that the α-amylase inhibition is concentration-dependent. IC_{50} values of P.E, $CHCl_3$, and EtOAc are 179.92, 226.80, and 355.66 µg/mL, respectively. When we compared the IC_{50} value of Acarbose (85.18 µg/mL) with the IC_{50} values of all the extracts, it was observed that the P.E extract showed the lowest IC_{50} value. Further, the % inhibition of P.E, $CHCl_3$, and EtOAc at 100 µg/mL was 27.79, 22.04, and 14.05%, respectively. The results obtained for Antioxidant activity of the extracts by the DPPH radical scavenging method were compared with Ascorbic acid and BHT and tabulated as shown in Table-5 and Fig.-2. Lower absorbance values correspond to higher free radical scavenging activity.

Table-5: Effect of Extracts on Radical Scavenging Activities by DPPH Method

Concentration (µg/mL)	% Inhibition				
	P. E	$CHCl_3$	EtOAc	AA	BHT
200	68.95	40.83	89.14	96.83	94.73
400	70.78	51.30	95.13	96.77	95.00
600	71.22	51.66	95.17	96.65	94.61
800	72.77	50.89	94.59	96.55	94.65
1000	72.70	51.44	95.14	96.46	94.73

From the Table-5, it is observed that P.E as well as Ethyl acetate extracts exhibit significant DPPH radical scavenging effect as compared to the standards, Ascorbic acid (AA) and BHT. The decreasing order of radical scavenging effect is EtOAc > P.E > $CHCl_3$. The Ethyl acetate extract shows no effect on glucoamylase and α-amylase inhibition but moderate DPPH radical scavenging activity.

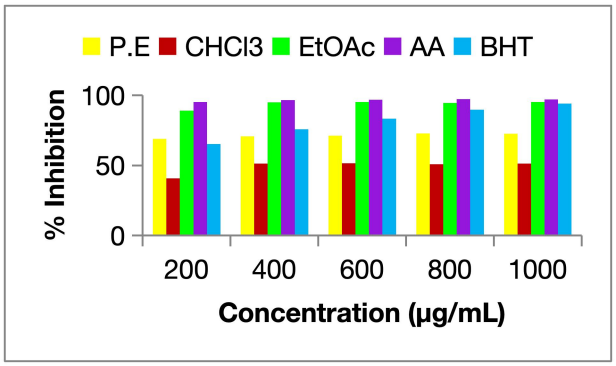


Fig.-2: Effect of Extracts on Radical Scavenging Activities by DPPH Method

Spectral Characterisation of Isolated Compound

Melting Point: 135-137°C.

Nature of the Compound: The compound appeared as a white crystalline solid.

GC-MS: 138[M^+],137,81,63,53

FT-IR: 3426 cm^{-1}, 3125 cm^{-1}, 2925.35 cm^{-1}, 2856.34 cm^{-1},1745.15 cm^{-1}, 1630.3 cm^{-1}, 1498.67 cm^{-1}, 1446.7 cm^{-1}, 1391.98 cm^{-1}, 1329.78 cm^{-1}.

^{1}H NMR: δ3.509 (1H, s (br), H-5), δ10.86 (1H, s (br), H-7), δ9.909 (1H, s, H-1), δ6.322 (1H, d,J=2.1, H-6), δ6.420 (1H, dd, J=8.4, H-4), δ 7.543 (1H, d,J=8.7, H-3).

^{13}C NMR: 191.091 (C1), 115.135 (C2), 132.4 (C3), 108.6 (C4), 165.14 (C5), 102.5 (C6), 163.2 (C7).

Interpretation of Spectra

Mass Spectra

A pure peak was obtained at retention time 11.70, indicating the presence of a single compound. The molecular peak was obtained at 138 m/z. The base peak of the compound was obtained at 137.07m/z. Other peaks obtained are at 81, 63, and 53 (Fig.-3).

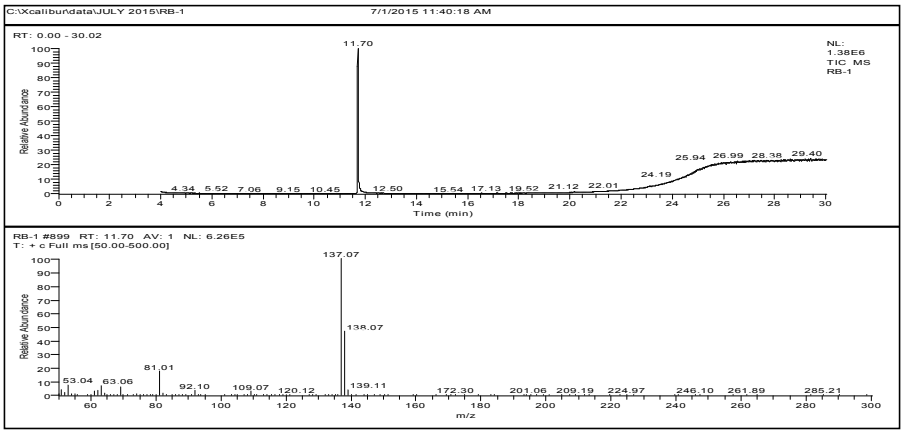


Fig.-3: GC-MS of Isolated compound

IR Spectrum

The IR spectrum shows an absorption band for the hydroxyl group at 3419.47 cm^{-1}. The absorption peak at 1687.92 cm-1 indicates the presence of a carboxyl group. The absorption bands at 1030.81 cm^{-1} and 996.19 cm^{-1} indicate the –C-OH, and another peak at 2923.75 cm^{-1} and 2825.55 cm^{-1} indicate the presence of aliphatic C-H stretching (Fig.-4).

^{1}H-NMR

The ^{1}H-NMR spectrum of the compound exhibited the presence of a singlet with a chemical shift at δ9.909 ppm, which was characteristic of the aldehyde proton (C-1). The compound shows a doublet at δ 7.543 (J= 8.7 Hz) (C-3). A doublet of doublets at δ 6.420 - δ 6.384 (J=8.7 & J=8.1) is due to interaction of C-3 and C-6 with C-4. A doublet was obtained at δ 6.329 (J=2.1) (C-6). A singlet was obtained at δ10.886 indicating the presence of a –OH group (Fig.-5).

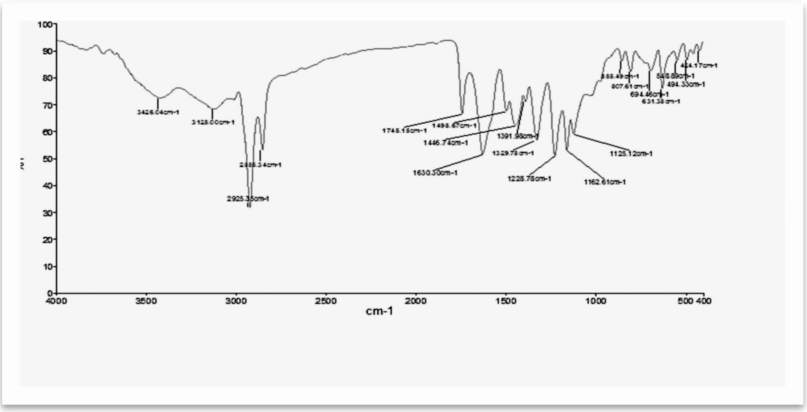


Fig.-4: FT-IR of Isolated Compound

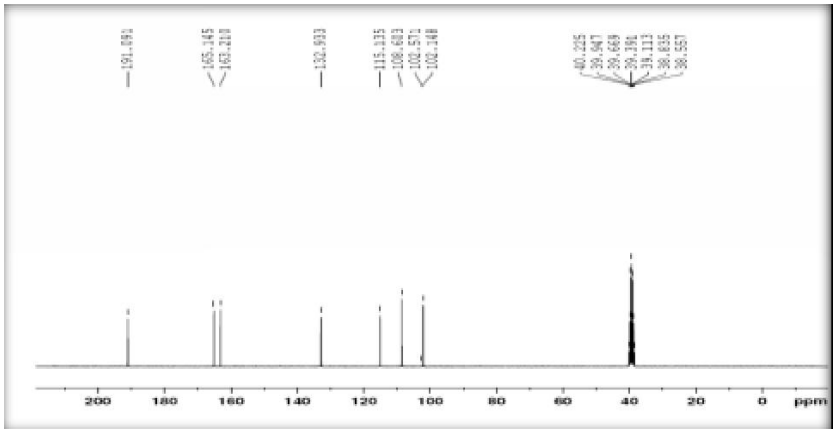


Fig.-5: ^{1}H NMR Spectra of Isolated Compound

^{13}C- NMR

^{13}C NMR spectrum of compound I exhibited a signal at δ 191.091, indicating the presence of a –CHO group. The spectral region of δ 165.145 and δ 163.210 indicated the presence of –OH groups in C-5 and C-7. The peaks at δ 132.933, δ 115.135, δ 108.603, δ 102.571 are present in the aromatic region (Fig.-6).

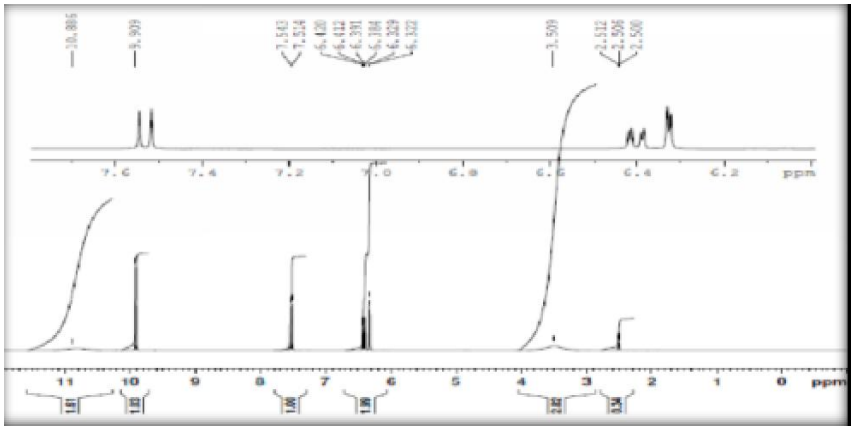


Fig.-6: ^{13}C –NMR Spectra of Isolated Compound

Based on the above spectral evidence, the isolated compound was identified to be β-Resorcyladehyde (Fig.-7).

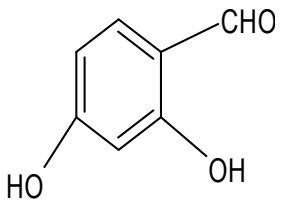


Fig.-7: The Isolated Compound Structure(β-Resorcyladehyde)

CONCLUSION

The present study paves the way for a novel method of the isolation of β-Resorcyladehyde, which shows no significant inhibitory activities against glucoamylase and α-amylase but moderate radical scavenging activity by the DPPH method. Literature review showed that Schiff bases of this compound are much more effective than the parent compound. Hence, synthesis of a novel Schiff base of this compound is a part of future study.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health & Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. W.L Mendelson & S. Hayden,, *Synthetic Communications*, **26(3)**, 603(1996), https://doi.org/10.1080/00397919608003654
2. V.A.Kumar, Y. Sarala, A.Siddikha, S.Vanitha, S. Babu, & A.V.Reddy, *Journal of Applied Pharmaceutical Science*,**8(4)**,1071(2018), https://dx.doi.org/10.7324/JAPS.2018.8410
3. T. A.Nibila, P. P Soufeena,P.Periyat, & K.K.Aravindakshan, *Journal of Molecular Structure*, 1231, 129938(2021), https://doi.org/10.1016/j.molstruc.2021.129938
4. J. Zheng, & L. Ma, *Bioorganic & Medicinal Chemistry Letters*, **25(10)**,2156(2015), https://doi.org/10.1016/j.bmcl.2015.03.078
5. M. S Al-Sharif, *International Journal of Electrochemical Science*, **18(12)**, 100384(2023), https://doi.org/10.1016/j.ijoes.2023.100384
6. A.N Taş, A.Şenocak, & A.Aydın, *Journal of the Turkish Chemical Society Section A: Chemistry*, **11(1)**,585(2024), https://izlik.org/JA83YX68DM
7. S.Murtaza, A.Abbas, K.Iftikhar, S.Shamim, M.S.Akhtar, Z.Razzaq & A.M.Elgorban, *Medicinal Chemistry Research*, **25**,2860(2016), https://doi.org/10.1007/s00044-016-1711-y
8. S.A.Aboafia, S.A.Elsayed, A.K.El-Sayed, & A.M.El-Hendawy, *Journal of Molecular Structure*,**1158**,39(2018), https://doi.org/10.1016/j.molstruc.2018.01.008
9. I.Kaya, & A. Avci, *Materials Chemistry and Physics*,**133(1)**,269(2012), https://doi.org/10.1016/j.matchemphys.2012.01.021

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