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Synthesis and Biological Evaluation of Some Novel Substituted N-Benzylideneaniline Derivatives

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ABSTRACT

N-benzylideneaniline is a class of important compounds in medicinal and pharmaceutical field. N-benzylideneaniline represents a model aromatic Schiff base and it is also a classic bioisoster of stilbene and resveratrol. Keeping in view the biological importance of N-benzylideneaniline we have synthesized some novel N-benzylideneaniline derivatives. The prepared compounds were tested for their in vitro antibacterial, antifungal and antioxidant activity. (4-fluoro-benzylidene)-(3,5-dichloro-phenyl)-amine **(5i)** showed in vitro antibacterial activity comparable to that of the standard Penicillin against *Escherichia coli*. The results of the in vitro antifungal activity showed that most of the synthesized derivatives have proven their antifungal potential. The results of the in vitro antioxidant tests showed that most of the synthesized compounds showed moderate (%RSA >50%) to mild (%RSA >40%) radical scavenging activity.

Key words: N-benzylideneaniline, Antibacterial activity, Antifungal activity, Antioxidant activity.

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INTRODUCTION

The frequency of life threaten infections such as tuberculosis, cancer, AIDS etc. caused by pathogenic microorganism is increasing worldwide and becoming an important cause of morbidity and mortality in immunocompromised patients. The synthesized compounds which are used for treatment of infectious diseases are known as chemotherapeutic. Every year thousands of compounds are synthesized with an aim to find potential chemotherapeutic agents to combat pathogenic microorganisms.

A majority of disease conditions like atherosclerosis, hypertension, ischemic diseases, Alzheimer's disease, Parkinsonism, cancer and inflammatory conditions are being considered caused primarily due to the imbalance between pro-oxidant and antioxidant homeostasis. Antioxidant principles from natural or Synthetic resources possess multifacetedness in their multitude and magnitude of activities and provide enormous scope in correcting the imbalance. Therefore, much attention is being directed to harness and harvest the antioxidant principles from synthetic resources. But very few compounds are withstood as therapeutic agent for various methodological tests. Antimicrobial, Antioxidant etc. are those activities required to perform for primary selection of compound as the therapeutic agents.

To overcome the alarming problem of microbial resistance to antibiotics, the discovery of a novel active compound against new targets is a matter of urgency. Many of the crude drugs, which are sources of medicinal preparations, still originate from wild-growing material. However, plant based drugs have shortened the life span of the source of material. There is a continuous search for more potent and cheaper raw materials.

The art of synthetic chemistry has always been a synthetic tool to design pharmacoactive compound, a wide screening, development of active analog and random discovery is a valuable and widely employed method for drug discovery. N-benzylideneanilines is one of these compounds and belongs to an important class of biologically active molecules. N-Benzylideneaniline are generally represented by the general formula $C_6H_5CH=NC_6H_5$ (5). The N-Benzylideneaniline are referred as "Imines, Anils, Azomethines, Schiff base etc. due to presence of C=N bond, the essential role of H-C=N linkage in the certain biological reactions prompted us to design different substituted N-Benzylideneaniline.

The previous study of N-benzylideneanilines reveals that it exhibits a wide spectrum of biological activities like antitubercular[1], antibacterial[2], antifungal[3], anticancer[4], anti-inflammatory[5], antioxidant and tyrosinase inhibitory activity[6]. This work concentrates on the design and synthesis of new analogues containing the n-benzylideneaniline (5) core as bioisoster of stilbene (1) and *trans*-resveratrol (2) (Fig 1).

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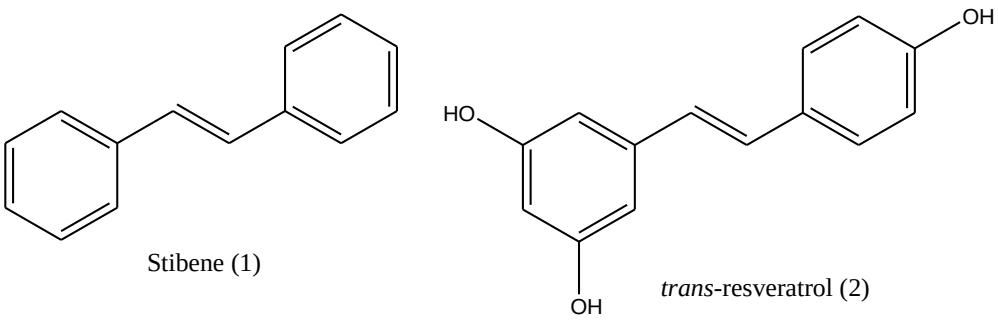
Figure 1. Structures of stilbene and *trans*-resveratrol



Stibene (1)

trans-resveratrol (2)

MATERIALS AND METHODS

General

All the chemicals used were procured from Aldrich, Spectrochem and Rankem Ltd. and purified using standard procedure if required. Melting points were recorded on an open capillary tube on Superfit melting point apparatus and are uncorrected. The purity of all the final compounds was assessed by thin layer chromatography (TLC). The Silica gel G was used for TLC. Completion of the reaction was monitored by TLC with petroleum ether:ethyl acetate (in varying proportion) system. TLC plates were visualized using iodine chamber. Structures of compounds were confirmed by IR, MASS and ^{1}H NMR spectra. IR spectra were recorded in KBr disk on JASCO FT-IR 5300. MASS spectra were recorded on SHIMADZU LC-MS 2010 EV Single qudrapole and are reported in ES-MS. ^{1}H NMR spectra were recorded on "BRUKER ADVANCE II 400 NMR Spectrophotometer" with tetramethylsilane (TMS) as the internal standard in $CDCl_3$.

General procedure for synthesis of N-benzylideneaniline derivatives

An equimolar quantity of benzaldehyde (1 mmol) and aniline (1 mmol) in toluene (5 ml) were heated to reflux in a Dean-stark apparatus. After the completion of reaction, the solvent was removed in *vaccuo* and the crude product was recrystallised from ethanol to give pure product. The precipitates of product were recrystallised at least three times to improve color and shape of crystals.

Synthesis of (4-Chloro-benzylidene)-(3-chloro-phenyl)-amine (5a)

Yield 48%. M. p.= 58-60^{o}C. FT-IR (KBr, cm^{-1}) ν_{max}: 3057, 1629, 1562, 670, 720. ES-MS: $C_{13}H_9Cl_2N$ [M+1] m/z, calcd 249, found 250.

Synthesis of (4-Chloro-phenyl)-(2,4-dichloro-benzylidene)-amine (5b)

Yield 31%. M. p.= 120-124 ^{o}C. FT-IR (KBr, cm^{-1}) ν_{max}: 3067, 1625, 1573, 650, 686. ^{1}H NMR δ: 7.18-7.17 (m, 2H), 7.36-7.33 (m, 3H), 7.45 (d, 1H), 8.19 (d, 1H), 8.82 (s, 1H). ES-MS: $C_{13}H_8Cl_3N$ [M+1] m/z, calcd 283, found 284.

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Synthesis of (3,5-Dichloro-benzylidene)-(2,4-dichloro-phenyl)-amine (5c)

Yield 31%. M. p.= 157-163 ^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3088, 1625, 1577, 670. ES-MS: $C_{13}H_7Cl_4N$ [M] m/z, calcd 317, found 317.

Synthesis of (4-Fluoro-benzylidene)-(4-fluoro-phenyl)-amine (5d)

Yield 60%. M. p.= 65-69^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3041, 2989, 1619, 1504, 1082. ^{1}H NMR δ: 7.03-7.01 (m, 2H), 7.09-7.05 (m, 4H), 7.84-7.81 (m, 2H), 8.33 (s, 1H); ES-MS: $C_{13}H_9F_2N$ [M+1] m/z, calcd 217, found 218.

Synthesis of (2,4-Difluoro-benzylidene)-(4-fluoro-phenyl)-amine (5e)

Yield 52 %. M. p.= 72-74^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3056, 3004, 1627, 1499, 1087. ^{1}H NMR δ: 6.94-6.91 (m, 1H), 6.98-6.98 (t, 1H), 7.08-7.06 (t, 2H), 7.22-7.21 (m, 2H), 8.17 (q, 1H), 8.68 (s, 1H); ES-MS: $C_{13}H_8F_3N$ [M+1] m/z, calcd 235, found 236.

Synthesis of (2,4-Difluoro-benzylidene)-(3,5-difluoro-phenyl)-amine (5f)

Yield 51%. M. p.= 73-76^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3065, 3021, 1624, 1498, 1129. ES-MS: $C_{13}H_7F_4N$ [M+1] m/z, calcd 253, found 254.

Synthesis of (4-Methyl-benzylidene)-p-tolyl-amine (5g)

Yield 68%. M. p.= 76-79^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3026, 2911, 1661, 1566, 1509. ^{1}H NMR δ: 2.37 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 7.19 (m, 4H), 7.26 (d, 2H), 7.79 (d, 2H), 8.43 (s, 1H). ES-MS : $C_{15}H_{15}N$ [M+1] m/z, calcd 209, found 210.

Synthesis of (3-Chloro-phenyl)-(4-fluoro-benzylidene)-amine (5h)

Yield 50%. FT-IR (KBr, cm^{-1}) v_{max}: 3075, 1661, 1566, 1509, 1192, 693. ES-MS: $C_{13}H_9ClFN$ [M] m/z, calcd 233, found 233.

Synthesis of (4-fluoro-benzylidene)-(3,5-dichloro-phenyl)-amine (5i)

Yield 70%. M. p.= 83-85^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3078, 2999, 1692, 1562, 1243, 1098, 708, 674. ^{1}H NMR δ: 7.20-7.14 (m, 4H), 7.87 (d, 3H), 8.35 (s, 1H). ES-MS: $C_{13}H_8Cl_2FN$ [M+1] m/z, calcd 267, found 268.

Synthesis of (4-Fluoro-benzylidene)-p-tolyl-amine (5j)

Yield 73%. M. p.= 62-64^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3026, 2880, 1629, 1502, 1291, 1223. ^{1}H NMR δ: 2.36 (s, 3H), 7.14-7.11 (m, 4H), 7.16 (t, 2H), 7.88 (m, 2H), 8.42 (s, 1H). ES-MS: $C_{14}H_{12}FN$ [M+1] m/z, calcd 213, found 214.

Synthesis of (4-Chloro-phenyl)-(4-methyl-benzylidene)-amine (5k)

Yield 61%. M. p.= 103-105^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3021, 2922, 1629, 1515, 716. ES-MS: $C_{14}H_{12}ClN$ [M+1] m/z, calcd 229, found 230.

Synthesis of (3,4-Dichloro-phenyl)-(4-methyl-benzylidene)-amine (5l)

Yield 58%. M. p.= 96-99^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3021, 2891, 1629, 1522, 718, 671. ES-MS: $C_{14}H_{11}Cl_2N$ [M+1] m/z, calcd 263, found 264.

Synthesis of (4-Fluoro-phenyl)-(4-methyl-benzylidene)-amine (5m)

Yield 58%. M. p.= 57-59^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3026, 2935, 1625, 1514, 1092, 1228. ^{1}H NMR δ: 2.41 (s, 3H), 7.07 (m, 2H), 7.18-7.16 (m, 2H), 7.26 (t, 2H), 7.77 (d, 2H), 8.39 (s, 1H). ES-MS: $C_{14}H_{12}FN$ [M+1] m/z, calcd 213, found 214.

Synthesis of (3,4-Difluoro-phenyl)-(4-methyl-benzylidene)-amine (5n)

Yield 57%. M. p.= 69-71^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3056, 2921, 1668, 1514, 1113, 1295. ES-MS: $C_{14}H_{11}F_2N$ [M] m/z, calcd 232, found 232.

Synthesis of (3,4-Dichloro-phenyl)-(3,4-dimethyl-benzylidene)-amine (5o)

Yield 53%. M. p.= 82-85^{o}C. FT-IR (KBr, cm^{-1}) v_{max}: 3073, 2955, 1618, 1562, 698. ^{1}H NMR δ: 2.34-2.33 (s, 6H), 7.04 (dd, 1H), 7.24 (d, 1H), 7.28 (d, 1H), 7.73 (dd, 1H), 7.68 (d, 1H), 8.34 (s, 1H). ES-MS: $C_{15}H_{13}Cl_2N$ [M+1] m/z, calcd 277, found 278.

***In- vitro* antibacterial activity** [7]

The agar cup plate method was used for the assessment of in vitro antibacterial activity of the synthesized compounds against *Escherishia coli* and *Staphylococcus aureus*. Penicillin was used as the standard of a clinically used antibacterial agent. The concentration of the compounds used was 2% solution. Drug-free controls were included and results are presented as zone of inhibition (mm). The values of zone of inhibition were determined after 24 hrs. of static incubation at 37^{o}C.

***In-vitro* antifungal activity** [8]

The poison plate method was used for the assessment of in vitro antifungal activity of the synthesized compounds against *Aspargillus niger* and *Penicilium chrysogenum*. Grysofulvin was used as the standard. The concentration of the compounds used was 2% solution. Drug-free controls were included and results are presented as the growth of organism observed or not. The growth of organism was determined after 2-3 days of static incubation at 30^{o}C.

***In-vitro* antioxidant activity** [9]

Free radical scavenging ability of the test compounds were determined by using the $DPPH^{\bullet}$ radical. An ethanol solution of $DPPH^{\bullet}$ (33mg in 1000 ml) was mixed with different concentration of each test compound (1000-2500 µg/ml) and the absorbance of $DPPH^{\bullet}$ (2,2-diphenyl-1-picryl-hydrazyl radical) change at 517 nm was measured 30 min later. Reaction solution without $DPPH^{\bullet}$ was used as blank and $DPPH^{\bullet}$ solution as control. Ascorbic acid was used as standard and results are mentioned as percentage radical scavenging activity.

RESULT AND DISCUSSION

Chemistry

N-benzylideneaniline derivatives were synthesized by condensation of substituted aniline with appropriate substituted benzaldehyde in Dean-stark apparatus [6]. (Scheme 1) The usual workup was followed by recrystallization from ethanol to give the corresponding N-benzylideneanilines (5a-5o).

Biological Evaluation

Antibacterial activity

All the synthesized compounds were tested for their in vitro antibacterial activity and activities of the compounds are shown in Table 1. All experiments were performed in comparison with Penicillin, a known antibacterial agent. Compound 5i was capable of showing an equipotent activity and other two compounds namely 5h and 5l showed moderate activity against *Escherichia coli*. While for remaining compounds failed to display any activity against the *Escherichia coli*. While for *Staphylococcus aureus* the activity was observed from moderate to mild antibacterial activity. Their zone of inhibition ranges from 8 mm to 22 mm.

Antifungal activity

The antifungal activities of the synthesized compounds are shown in Table 2. All experiments were performed in comparison with Grysofulvin, a known antifungal agent. Compounds 5a, 5c, 5d, 5h, 5i and 5k-5o have displayed antifungal activity against *Aspergillus*

niger. All the compounds except 5e and 5g inhibited the growth of *Penicillium chrysogenum* and have proved their antifungal potential.

Antioxidant activity

The antioxidant activities of the compounds are shown in Table 3. The DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) radical scavenging effect was carried out according to the method first employed by Blois. The 200 mL of sample solution was added to 300 mL of DPPH$^{\bullet}$ solution in ethanol. After incubation at room temperature for 30 min, the absorbance of this solution was determined at 517 nm using a spectrophotometer and the remaining DPPH was calculated. All experiments were carried out in triplicate and repeated at least three times. All experiments were performed in comparison with Ascorbic acid, a known antioxidant agent. Results are expressed as percentage decrease with respect to control values. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula.

$$\% \text{ Radical scavenging activity} = \frac{\text{Control Abs} - \text{Sample Abs}}{\text{Control Abs}} \times 100$$

The antioxidant activity was evaluated by DPPH free radical scavenging assay and compounds have shown moderate (%RSA>40) to significant (%RSA>50) radical scavenging activity.

Table 1. Antibacterial activity of N-benzylideneaniline derivatives compared with the standard Penicillin

Sr. No.	*E. coli*	*S. aureus*
5a	-ve	-ve
5b	-ve	-ve
5c	-ve	-ve
5d	-ve	-ve
5e	-ve	-ve
5f	-ve	16 mm
5g	-ve	8 mm
5h	13 mm	12 mm
5i	18 mm	22 mm
5j	-ve	-ve
5k	-ve	-ve
5l	12 mm	17 mm
5m	-ve	-ve
5n	-ve	-ve
5o	-ve	12 mm
DMSO	-ve	-ve
Std	17 mm	40 mm

DMSO = Dimethyl sulfoxide
-ve = No antibacterial activity observed
Zone of inhibition in millimeters **(**mm), **Std** = Penicillin

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Table 2. Antifungal activity of N-benzylideneaniline derivatives compared with the standard Griseofulvin

Sr. No.	*A. niger*	*P. chrysogunum*
5a	-ve	-ve
5b	+ve	-ve
5c	-ve	-ve
5d	+ve	-ve
5e	+ve	+ve
5f	-ve	-ve
5g	+ve	+ve
5h	-ve	-ve
5i	-ve	-ve
5j	+ve	-ve
5k	-ve	-ve
5l	-ve	-ve
5m	-ve	-ve
5n	-ve	-ve
5o	-ve	-ve
DMSO	+ve	+ve
Std	-ve	-ve

DMSO= Dimethylsulfoxide
+ve = No antifungal activity observed
-ve = Antifungal Activity observed
Std = Griseofulvin

Table 3. % Radical scavenging activity of N-benzylideneaniline derivatives compared with the standard Ascorbic acid

Sl. No	Conc. (µg/ml)	Abs.	DPPH Abs.	% RSA	Std. Deviation
5a	2500	0.123	0.5990	79.47	±1.01
	1500	0.240	0.5990	59.99	±0.75
	1000	0.267	0.5990	55.37	±0.67
5b	2500	0.176	0.5990	70.67	±0.51
	1500	0.227	0.5990	62.10	±1.10
	1000	0.266	0.5990	55.65	±0.42
5c	2500	0.244	0.5990	59.27	±0.44
	1500	0.253	0.5990	57.76	±0.17
	1000	0.256	0.5990	57.26	±0.34
5d	2500	0.285	0.5330	46.53	±0.75
	1500	0.293	0.5330	45.03	±1.50
	1000	0.297	0.5330	44.22	±0.29
5e	2500	0.265	0.5330	50.22	±0.66
	1500	0.275	0.5330	48.47	±0.76
	1000	0.288	0.5330	46.03	±0.66

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5f	2500	0.261	0.5330	51.09	±0.85
	1500	0.276	0.5330	48.22	±0.57
	1000	0.280	0.5330	47.47	±0.75
5g	2500	0.191	0.5330	64.17	±1.13
	1500	0.257	0.5330	51.84	±0.66
	1000	0.307	0.5330	42.40	±0.38
5h	2500	0.239	0.5990	60.10	±1.00
	1500	0.255	0.5990	57.48	±0.20
	1000	0.276	0.5990	53.98	±0.59
5i	2500	0.297	0.5330	44.28	±0.38
	1500	0.310	0.5330	41.78	±0.85
	1000	0.321	0.5330	39.71	±0.47
5j	2500	0.191	0.5330	64.10	±0.85
	1500	0.217	0.5330	59.22	±0.95
	1000	0.255	0.5330	52.16	±0.19
5k	2500	0.225	0.5330	57.85	±0.39
	1500	0.239	0.5330	55.10	±0.47
	1000	0.256	0.5330	51.97	±0.19
5l	2500	0.276	0.5330	48.16	±0.58
	1500	0.292	0.5330	45.15	±0.11
	1000	0.299	0.5330	43.96	±0.39
5m	2500	0.286	0.5330	46.34	±0.56
	1500	0.289	0.5330	45.72	±1.04
	1000	0.292	0.5330	45.22	±1.13
5n	2500	0.278	0.5330	47.84	±0.19
	1500	0.295	0.5330	44.72	±0.60
	1000	0.315	0.5330	40.96	±0.57
5o	2500	0.191	0.5330	64.10	±0.63
	1500	0.256	0.5330	52.03	±0.13
	1000	0.307	0.5330	42.46	±0.34
	50	0.020	0.731	97.26	±0.47
	40	0.030	0.731	95.90	±0.36
AA	30	0.050	0.731	93.16	±0.36
	20	0.072	0.731	90.15	±0.36
	10	0.118	0.731	83.86	±0.60

% RSA: Percentage Radical Scavenging Activity.
Std. Deviation: Standard deviation
Conc.: Concentration
Abs.: Absorbance
AA: Ascorbic acid

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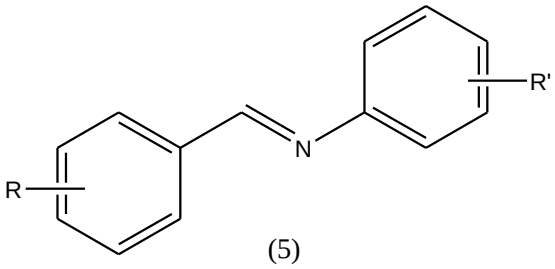
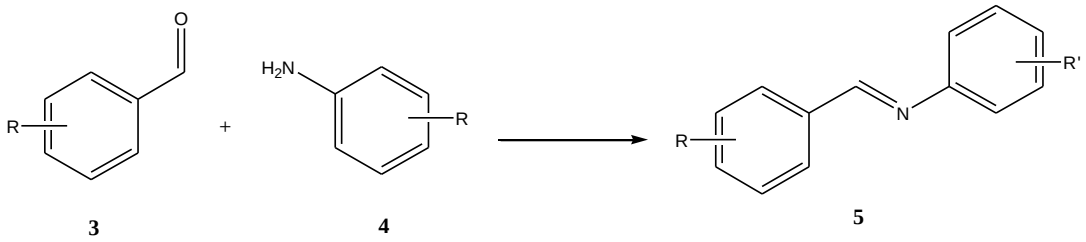
Figure 2. Designed general structure of substituted N-benzylideneaniline (5) as isoster of 1 and 2



(5)

Scheme 1: Synthesis of compounds 5a-5o



3 4 5

Reagents and conditions: (a) Toluene; (b) Reflux for 16 hrs. in Dean-stark apparatus.

CONCLUSION

The synthesized novel N-benzylideneaniline derivatives have been characterized by using IR, Mass and ^{1}HNMR spectroscopy. The synthesized N-benzylideneaniline derivatives were evaluated for their in vitro antibacterial, antifungal and antioxidant potential. The antibacterial activity was evaluated against *E. coli* and *S. aureus* using agar cup plate method. Among the tested compounds, (4-fluoro-benzylidene)-(3,5-dichloro-phenyl)-amine (5i**)** showed equipotent antibacterial activity against *E. coli* (18 mm) and moderate antibacterial activity against *S. aureus* as compared to penicillin (17 mm and 40 mm). Compounds namely 5f-h**,** 5l and 5o have shown mild antibacterial activity against *S. aureus*.

The antifungal activity was evaluated against *A. niger* and *P. chrysogenum* using poison plate method. Compounds 5a**,** 5c, 5f, 5h, 5i, 5k-o have shown positive antifungal activity against *A. niger*. All Compounds except 5e and 5g inhibited the growth of *P. chrysogenum* and have proved their antifungal potential. The antioxidant activity was evaluated by DPPH free radical scavenging assay and compounds have shown moderate (%RSA>40) to significant (%RSA>50) radical scavenging activity.

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