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Synthesis and pharmacological studies of some 1-(substituted benzylidine)-2-(2-carbethoxy-4-nitrophenyl)hydrazines

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ABSTRACT

An exclusive nucleophillic substitution reaction occurred resulting in the formation of 2-carbethoxy-4-nitrophenylhydrazine from 2-chloro-5-nitroethylbenzoate rather than expected 2-chloro-5-nitrobenzhydrazide. The 2-carbethoxy-4-nitrophenyl when treated with suitably substituted aldehydes gave corresponding 1-(substituted benzylidine)-2-(2-carbethoxy-4-nitrophenyl)hydrazines. Structures of the newly synthesized compounds were established on the basis of spectral and analytical data. The new compounds were also evaluated for their in vitro antioxidant, antibacterial and antifungal activity.

Keywords: Nucleophillic substitution reaction, Hydrazones, antioxidant property, antibacterial, antifungal activity

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INTRODUCTION

Hydrazones belong to the important class of compounds because of their diverse biological and clinical applications. Hydrazones possessing an azometine -NHN=CH- moiety constitute an important class of compounds for new drug development. This created interest in researchers who have synthesized these compounds as target structures and evaluated their biological activities [1-3]. They possess anti microbial [4], anti inflammatory [5], anticancer [6] activity.

Prompted by these observations and in continuation of our work on biologically potential heterocycles [7-12] we herein report the synthesis of a series of 1-(substituted benzylidine)-2-(2-carbethoxy-4-nitrophenyl)hydrazines and their pharmacological activity.

MATERIALS AND METHOD

Melting points were determined in open capillary tubes and are uncorrected. Elemental analysis was carried out in Vario EL III Elementa model. IR spectra were recorded by dispersing the compounds in KBr pellets on a Schimadzu FT-IR 157 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker Avance II 400 MHz NMR spectrometer and all the chemical shift values were reported as δ (ppm). Mass spectra were recorded on LC/MS system JEOL JMS – D300 mass spectrometer operating at 70eV. Purity of the newly synthesised compounds was checked by TLC on silica gel plates (Merck, Silica gel 60F254).

Synthesis of ethyl-2-chloro-5-nitrobenzoate (2)
2-Chloro-5-nitro-benzoic acid (**1**) was procured from Sigma-Aldrich (Product No. 125113). This compound (20.1g, 0.1 mol) was taken in absolute alcohol (60mL, 1 mol) and Conc. Sulphuric acid (1 mL) in a 250 mL round bottomed flask and refluxed on a water bath for 8 hours. Excess of the solvent was removed under reduced pressure. Contents were then washed with sodium bicarbonate solution to remove any acid impurity and pure compound was collected. M. p. 30-32 °C, yield 89 %.

Synthesis of 2-carbethoxy-4-nitrophenylhydrazine (4)
Ethyl-2-chloro-5-nitrobenzoate (0.05mol) and hydrazine hydrate (99 %, 0.05 mol) were taken in a round bottomed flask equipped with reflux condenser. The contents were refluxed on a water bath for 6 hours. Precipitated 2-carbethoxy-4-nitrophenylhydrazine was filtered, dried and recrystallised using ethanol. M. p. 120 °C, 76 %.

Mol. Formula: $C_9H_{11}N_3O_4$; Elemental analysis (Found): C, 47.96; H, 4.92; N, 18.54; (calculated) C, 48.0; H, 4.86; N, 18.66; IR (KBr) γ/cm^{-1}: 3106.6 (N-H), 2985.8 (C-H), 1689.5 (Ester C=O), 1565.3 (Asym. N=O), 1329.7 (Sym. N=O). ^{1}H NMR (DMSO-d_6) δ: 1.1 (t, 3H, CH_3), 3.5 (s, 1H, NH), 3.71 (q, 2H, CH_2), 4.1 (br, 2H, NH_2), 7.18- 8.4 (m, 3H, Ar-H); MS: m/z = 226 [M^+ +1].

Synthesis of 1-(substituted benzylidine)-2-(2-carbethoxy-4-nitrophenyl)hydrazines (5a-h)
2-Carbethoxy-4-nitrophenylhydrazine (0.01 mol) and substituted benzaldehyde (0.01 mol) was dissolved in ethanol (10 mL). Cooled and added 3 drops of Conc. H_2SO_4. Contents were then heated under reflux on a water bath. Completion of the reaction was monitored by TLC. Filtered the solid obtained, dried and recrystallised from ethanol to give compounds 5a-h.

1-(4-Dimethylaminobenzylidene)-2-(2-carbethoxy-4-nitrophenyl)hydrazines (5a)
R= $N(CH_3)_2$, R_1= H; M. p. 164-66 °C, yield 71 %; Mol. Formula: $C_{18}H_{20}N_4O_4$; Elemental analysis (Found): C, 60.56; H, 5.52; N, 15.64; (calculated) C, 60.66; H, 5.66; N, 15.72; IR (KBr) γ/cm^{-1}: 3240.6 (N-H), 2918.9 (C-H), 1682.5 (C=N), 1597.9 (Asym. N=O), 1324.2 (Sym. N=O). ^{1}H NMR (DMSO-d_6) δ: 1.37 (t, 3H, J= 7.03 Hz, CH_3), 2.97 (s, 6H, $N(CH_3)_2$), 4.38 (q, 2H, J= 6.96 Hz, CH_2), 6.74- 8.24 (m, 7H, Ar-H), 8.64 (s, 1H, NH), 11.35 (s, 1H, CH=N); MS: m/z = 357 [M^+ +1].

1-(Benzylidine)-2-(2-carbethoxy-4-nitrophenyl)hydrazines (5b)
R= H, R_1= H; M. p. 169-170 °C, yield 68 %; Mol. Formula: $C_{16}H_{15}N_3O_4$; Elemental analysis (Found): C, 61.25; H, 4.76; N, 13.36; (calculated) C, 61.34; H, 4.83; N, 13.41; IR (KBr) γ/cm^{-1}: 3232.1 (N-H), 2991.5 (C-H), 1676.7 (C=N), 1577.6 (Asym. N=O), 1321.9 (Sym. N=O). ^{1}H NMR (DMSO-d_6) δ: 1.37 (t, 3H, J= 7.08 Hz, CH_3), 4.39 (q, 2H, J= 7.52 Hz, CH_2), 7.42- 8.65 (m, 8H, Ar-H), 8.66 (s, 1H, NH), 11.51 (s, 1H, CH=N); MS: m/z = 314 [M^+ +1].

1-(4-Nitrobenzylidine)-2-(2-carbethoxy-4-nitrophenyl)hydrazines (5c)
R= NO_2, R_1= H; M. p. 224 °C, yield 65 %; Mol. Formula: $C_{15}H_{12}N_4O_6$; Elemental analysis (Found): C, 52.26; H, 3.44; N, 16.19; (calculated) C, 52.33; H, 3.51; N, 16.27; IR (KBr) γ/cm^{-1}: 3227.9 (N-H), 2918.6 (C-H), 1691.3 (C=N), 1508.8 (Asym. N=O), 1327.6 (Sym. N=O). ^{1}H NMR (DMSO-d_6) *δ*: 1.39 (t, 3H, *J*= 6.95 Hz, CH_3), 4.42 (q, 2H, *J*= 7.04 Hz, CH_2), 7.51- 8.69 (m, 8H, Ar-H), 8.71 (s, 1H, NH), 11.65 (s, 1H, CH=N); MS: m/z = 345 [M^+ +1].

1-(4-Chlorobenzylidine)-2-(2-carbethoxy-4-nitrophenyl)hydrazines (5d)
R= Cl, R_1= H; M. p. 194 °C, yield 72 %; Mol. Formula: $C_{16}H_{14}ClN_3O_4$; Elemental analysis (Found): C, 55.19; H, 4.01; N, 11.98; (calculated) C, 55.26; H, 4.06; N, 12.08; IR (KBr) γ/cm^{-1}: 3186.1 (N-H), 2941.7(C-H), 1688.1 (C=N), 1518.6 (Asym. N=O), 1338.6 (Sym. N=O). ^{1}H NMR (DMSO-d_6) *δ*: 1.37 (t, 3H, *J*= 7.12 Hz, CH_3), 4.39 (q, 2H, *J*= 6.94 Hz, CH_2), 7.48- 8.66 (m, 8H, Ar-H), 8.69 (s, 1H, NH), 11.58 (s, 1H, CH=N); MS: m/z = 348.5 [M^+ +1].

1-(4-Hydroxybenzylidine)-2-(2-carbethoxy-4-nitrophenyl)hydrazines (5e)
R= OH, R_1= H; M. p. 214-15 °C, yield 68 %; Mol. Formula: $C_{16}H_{15}N_3O_5$; Elemental analysis (Found): C, 58.27; H, 4.47; N, 12.69; (calculated) C, 58.36; H, 4.59; N, 12.76; IR (KBr) γ/cm^{-1}: 3198.1 (N-H), 2964.1 (C-H), 1665.1 (C=N), 1563.7 (Asym. N=O), 1338.8 (Sym. N=O). ^{1}H NMR (DMSO-d_6) *δ*: 1.36 (t, 3H, *J*= 6.88 Hz, CH_3), 4.38 (q, 2H, *J*= 6.96 Hz, CH_2), 7.54- 8.32 (m, 8H, Ar-H), 8.67 (s, 1H, NH), 11.38 (s, 1H, CH=N); MS: m/z = 330 [M^+ +1].

1-(4-Methoxybenzylidine)-2-(2-carbethoxy-4-nitrophenyl)hydrazines (5f)
R= OCH_3, R_1= H; M. p. 179 °C, yield 76 %; Mol. Formula: $C_{17}H_{17}N_3O_5$; Elemental analysis (Found): C, 59.41; H, 4.91; N, 12.19; (calculated) C, 59.47; H, 4.99; N, 12.24; IR (KBr) γ/cm^{-1}: 3209.1 (N-H), 2969.3 (C-H), 1683.8 (C=N), 1576.2 (Asym. N=O), 1341.5 (Sym. N=O). ^{1}H NMR (DMSO-d_6) *δ*: 1.37 (t, 3H, *J*= 7.64 Hz, CH_3), 3.87 (s, 3H, OCH_3), 4.36 (q, 2H, *J*= 7.12 Hz, CH_2), 7.36- 8.46 (m, 7H, Ar-H), 8.64 (s, 1H, NH), 11.34 (s, 1H, CH=N); MS: m/z = 344 [M^+ +1].

1-(4-Hydroxy-3-methoxybenzylidene)-2-(2-carbethoxy-4-nitrophenyl)hydrazines (5g)
R= OH, R_1= OCH_3; M. p. 197 °C, yield 66 %; Mol. Formula: $C_{17}H_{17}N_3O_6$; Elemental analysis (Found): C, 56.76; H, 4.71; N, 11.59; (calculated) C, 56.82; H, 4.77; N, 11.69; IR (KBr) γ/cm^{-1}: 3231.5 (N-H), 2993.7 (C-H), 1671.6 (C=N), 1580.6 (Asym. N=O), 1328.1 (Sym. N=O). ^{1}H NMR (DMSO-d_6) *δ*: 1.35 (t, 3H, *J*= 6.92 Hz, CH_3), 3.85 (s, 3H, OCH_3), 4.35 (q, 2H, *J*= 7.08 Hz, CH_2), 7.18- 8.5 (m, 7H, Ar-H), 8.65 (s, 1H, NH), 11.37 (s, 1H, CH=N); MS: m/z = 360 [M^+ +1].

1-(3, 4-Dimethoxybenzylidene)-2-(2-carbethoxy-4-nitrophenyl)hydrazines (5h)
R= OCH_3, R_1= OCH_3; M. p. 186 °C, yield 71 %; Mol. Formula: $C_{18}H_{19}N_3O_6$; Elemental analysis (Found): C, 57.81; H, 5.04; N, 11.19; (calculated) C, 57.90; H, 5.13; N, 11.25; IR (KBr) γ/cm^{-1}: 3205.9 (N-H), 2978.1 (C-H), 1691.8 (C=N), 1564.1 (Asym. N=O), 1334.1 (Sym. N=O). ^{1}H NMR (DMSO-d_6) *δ*: 1.36 (t, 3H, *J*= 7.1 Hz, CH_3), 3.89 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.37 (q, 2H, *J*= 6.64 Hz, CH_2), 7.21- 8.43 (m, 6H, Ar-H), 8.67 (s, 1H, NH), 11.38 (s, 1H, CH=N); MS: m/z = 374 [M^+ +1].

Biological Evaluation

DPPH radical scavenging activity

Williams's procedure [13] was followed for evaluation of the free radical-scavenging capacity. Briefly, 1 mL of the test sample (100 µg/mL) was mixed with the methanolic 2,2-diphenyl-1-

picrylhydrazyl (DPPH) solution (2 mL, 0.2 mM). The absorbance was measured at 517 nm immediately after standing at room temperature for 30 min. The percentage of scavenging has been calculated as the ratio of the absorption of the sample relative to the control DPPH (0.2 mM) solution without the test samples. DPPH radical-scavenging activity was expressed as the inhibition percentage. Results are shown in **Table 1**.

Table 1: DPPH radical scavenging assay for the compounds 5a-h

Compd No	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**BHT**
% Inhibition	71.27	78.43	78.38	78.29	75.39	72.48	77.82	78.51	94.87

Antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* using Agar well diffusion method [14]. In this method, 24 hours old Muller- Hinton broth cultures of test bacteria were swabbed uniformly on solidified sterile Muller-Hinton agar plates using sterile cotton swab. Then, aseptically wells of 6 mm diameter were bored in the inoculated plates with the help of gel puncher and the samples (100 µL), standard (Amoxicillin, 100 µL) and control (DMSO) were added into the labelled wells. The plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition was recorded. Experiments were done at concentrations of 100 and 50 µg/mL of the test samples. The results are summarised in **Table-1**.

Table 2: Antibacterial activity of compounds 5a-h

Compd	Diameter of zone of inhibition (mm)					
	E. coli		***P. aeruginosa***		***S. aureus***	
	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL
5a	15	12	15	12	10	05
5b	14	09	14	11	12	06
5c	17	13	17	11	15	09
5d	17	15	17	12	15	11
5e	15	13	17	10	13	10
5f	12	05	11	06	11	06
5g	14	10	15	11	15	11
5h	16	10	14	10	11	07
Standard	17	14	16	12	14	10
Control	0	0	0	0	0	0

Antifungal activity

The antifungal activity was carried out against *Candida albicans* and *Aspergillus flavus* by agar well diffusion method [15]. The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02ml) of inoculum was introduced to molten PDA and poured in to a petri dish. After solidification, the appropriate wells were made on agar plate by using cork borer. Incubation period of 24- 48 hours at 28 °C was maintained for observation of antifungal activity of the compounds. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth. Metronidazole was used as standard. The complete antifungal analysis was carried out under strict aseptic conditions. The results are summarized in **Table-3**.

Table 3: antifungal activity of compound 5a-h

Compd	Diameter of zone of inhibition (mm)			
	C. albicans		***A. flavus***	
	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL
5a	17	14	15	10
5b	15	11	15	11
5c	19	14	16	12
5d	19	14	17	14
5e	17	12	15	09
5f	12	09	14	11
5g	15	10	13	06
5h	15	09	12	08
Standard	20	16	16	13
Control	0	0	0	0

RESULTS AND DISCUSSION

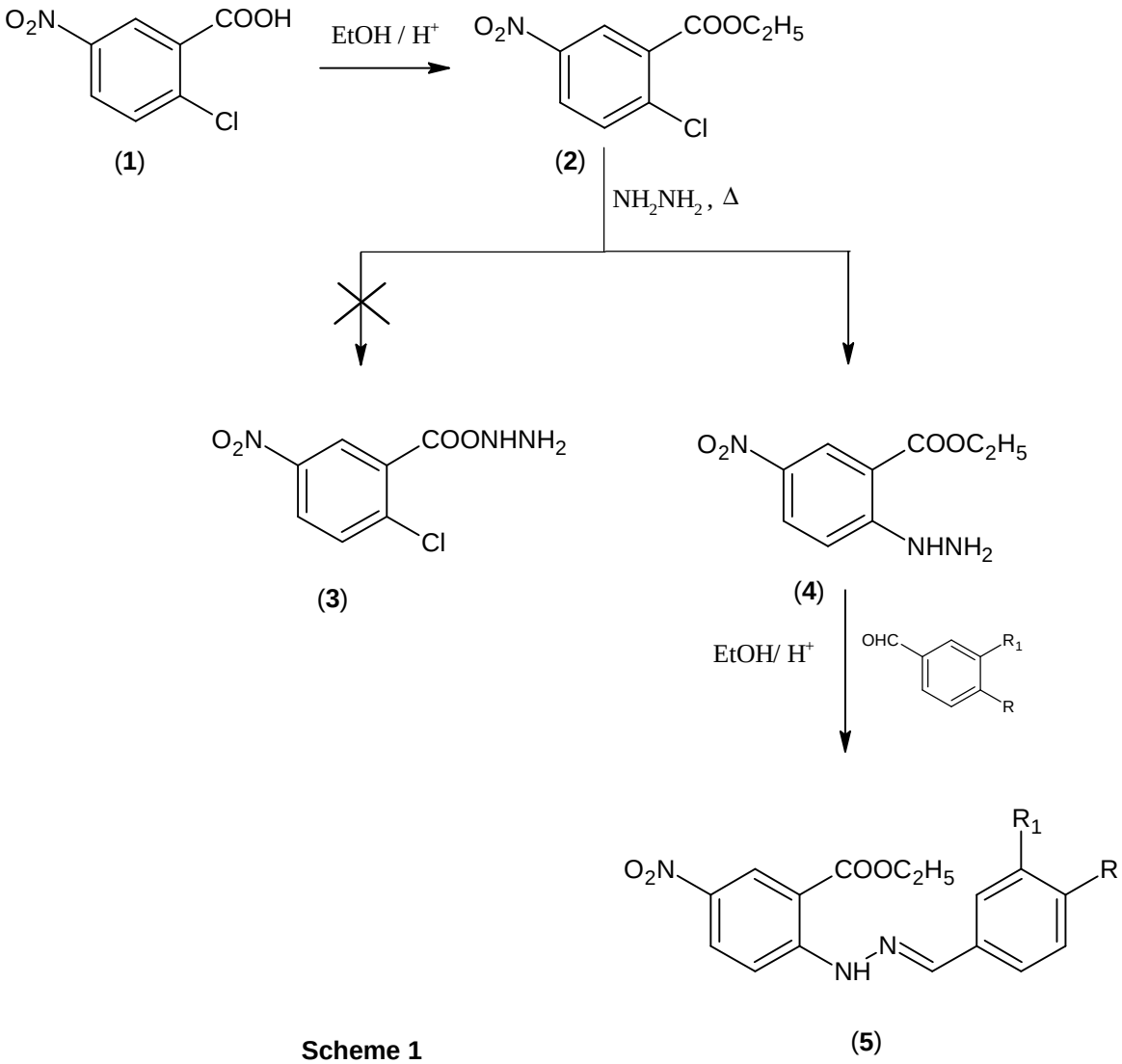
Chemistry

One of the very interesting observations in this reaction is the exclusive formation of 2-Carbethoxy-4-nitrophenylhydrazine (**4**). When ethyl-2-chloro-5-nitrobenzoate (**2**) was made to undergo hydrazinolysis, it can either undergo condensation reaction leading to the formation of 2-chloro-5-nitrobenzhydrazide (**3**) or it can undergo nucleophillic substitution resulting in the formation of 2-carbethoxy-4-nitrophenylhydrazine (**4**). During the present study it was observed that when the reaction was carried out with equimolar amounts of ethyl-2-chloro-5-nitrobenzoate (**2**) and hydrazine, 2-Carbethoxy-4-nitrophenylhydrazine (**4**) is obtained as the sole product with high yield.

Pharmacology

Test samples **5a-h** were screened for their free radical scavenging activity by DPPH method and the results are depicted in **Table 1**. Antioxidant reacts with DPPH, which is a stable free radical and converts it to 1,1- diphenyl-2-picrylhydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant compounds. Among the compounds tested all the compound showed significant antioxidant property when compared with standard butylated hydroxytoluene (BHT).

When compounds **5a-h** were screened for antibacterial and anti fungal activity encouraging results have been obtained (**Table 2** and **Table 3**). All the compounds tested showed good inhibition of growth of the bacteria *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These compounds were also showed potent inhibition of *Candida albicans* and *Aspergillus flavus*. Compounds **5c** and **5d** showed the highest inhibitory effect against all the tested organisms. Looking at the results it can be concluded the antibacterial and antifungal activities of some of the compounds are comparable to positive controls.



(5)

Scheme 1

CONCLUSION

In conclusion we herein report the exclusive formation of 2-carbethoxy-4-nitrophenylhydrazine (**4**) with high yield and purity. This (**4**) was converted in to 1-(substituted benzylidine)-2-(2-carbethoxy-4-nitrophenyl)hydrazines (**5a-h**) by the reaction with suitably substituted benzaldehydes.

Furthermore the results obtained from antioxidant, antifungal and antibacterial screening of the novel compounds is encouraging. Most of the compounds tested showed significant pharmacological activities when compared to standard. Thus on the basis of the results obtained it can be concluded that the 1-(substituted benzylidine)-2-(2-carbethoxy-4-nitrophenyl)hydrazines demonstrated good antioxidant, antibacterial and antifungal activity.

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REFERENCE

[1] K. K. Sivakumar, A. Rajasekaran, I. Ponnilavarasan, A. Somasundaram, R. Sivasakthi , S. Kamalaveni, *Der Pharmacia Lettre*, **2010**, 2, 211-219.
[2] S. Rollas, Ş. G. Küçükgüzel, *Molecules*, **2007**, 12, 1910-1939.
[3] B. Narasimhan, P. Kumar, D. Sharma, *Acta Pharma. Sci.*, **2010**, 52, 169-180.
[4] O. O. Ajani, C. A. Obafemi, O. C. Nwinyi, D. A. Akinpelu, *Bioorg. Med. Chem.*, **2010**, 18, 214-221.
[5] M. Gökçe, S. Utku, E. Küpeli, *Eur. J. Med. Chem.*, **2009**, 44, 3760-3764.
[6] J. Easmon, G. Puerstinger, T. Roth, H. H. Fiebig, M. Jenny, W. Jaeger, G. Heinisch, J. Hofmann, *Int. J. Cancer*, **2001**, 94, 89–96.
[7] K. V. Sujith, J. N. Rao, P. Shetty, B. Kalluraya, *Eur. J. Med. Chem*. **2009**, 44, 3697-3702.
[8] A. M. Isloor, B. Kalluraya, P. Shetty, *Eur. J. Med. Chem*. **2009**, 44, 3784-3787.
[9] A. M. Isloor, B. Kalluraya, K.S. Pai, *Eur. J. Med. Chem*. **2010**, 45, 825-830.
[10] K. S .Girish, B. Kalluraya, V. Narayana, Padmashree, *Eur. J. Med. Chem*. **2010**, 45, 4640-4644.
[11] G.C. Ramaprasad, B. Kalluraya, B. Sunil Kumar, R. K. Hunnur, *Eur. J. Med. Chem*. **2010**, 45, 4587-4593.
[12] J. Gowda, A. M. A. K. Khadar, B. Kalluraya, N. S. Kumari, *Indian J. Chem*. **2010**, 49B, 1130-1134.
[13] W. B. Williams, M. E. Cuvelier, C. Berset,. *Lebensm.-Wiss. Technol.*, **1995**, 28, 25-30.
[14] B. Tepe, E. Donmez, M. Unlu, F. Candan, D. Daferera, G. M. Vardar-Unlu Polissiou, A. Sokmen, *Food Chemistry*, **2004**, 84, 519-525.
[15] C. Perez, A. Pauli, P. Bazerque, *Acta Biol. Med. Exp*., **1990**, 15, 113-115.