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Synthesis, characterization and biological evaluation of some pyrazoles derived from *α,β*-dibromo 4,4'-difluoro chalcone

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ABSTRACT

Pyrazole derivatives are prepared by condensing α,β- dibromo 4,4'-difluoro chalcone with various hydrazine derivatives. All these derivatives are characterized by NMR, IR and mass spectral data. All the synthesized products are screened for their in vitro antimicrobial and antioxidant properties. Some of the tested compounds exhibited significant DPPH scavenging activity.

Keywords : *α,β*-Dibromo 4,4'-difluoro chalcone, Pyrazole, Antimicrobial, DPPH scavenging assay.

INTRODUCTION

Pyrazole derivatives are well-known and important nitrogen-containing five-membered heterocyclic compounds and have occupied a unique position in the design and synthesis of novel biologically active agents. They display various biological activities such as antitumor, antibacterial, antifungal, antiviral, antiparasitic, anti-tubercular and insecticidal [1-3]. Some of these compounds also possess antioxidant, anti-inflammatory and analgesic properties [4,5]. Further, pyrazole derivatives are also used as chelating agents [6] and inhibitors for the corrosion of the steel [7]. Due to these interesting activities of pyrazole derivatives, considerable attention has been focused on this class of compounds. In addition, pyrazoles have played a crucial part in the development of theory in heterocyclic chemistry and also used extensively in organic synthesis [8].

α,β-Dibromo chalcones are valuable synthetic building blocks, which are employed for several types of synthetic transformations, in particular for synthesis of heterocyclic compounds such as pyrazoles and hydroxy pyrazoline derivatives with antibacterial and antifungal activities [9, 10]. Hence, in view of the importance of pyrazole derivatives and in continuation of our work on synthesis of various derivatives of 4,4'-difluoro chalcone [11-21], it was decided to prepare pyrazole derivatives by using 4,4'-difluoro chalcone dibromide as the starting material and to study their biological activities.

MATERIALS AND METHODS

2.1. Chemistry

Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using Merck silica gel 60 F_{254} coated aluminium plates. IR spectra were recorded on Shimadzu-FTIR Infrared spectrometer in KBr (ν_{max} in cm^{-1}). ^1H NMR (400 MHz) spectra were recorded on a Bruker AMX 400 spectrometer, with 5mm PABBO BB -1H TUBES with TMS as internal standard. LCMS were obtained using Agilent 1200 series LC and Micromass zQ spectrometer. Elemental analysis was carried out by using VARIO EL-III (Elementar Analysensysteme GmBH).

2.1.1. General procedures for the synthesis

Synthesis of 2,3-dibromo-1,3-bis(4-fluorophenyl)propan-1-one (2)

To a solution of 4,4'-difluoro chalcone **1** (24.4 g, 0.1 mol) in acetic acid (100 mL), bromine (20.0 g, 0.125 mol) in acetic acid (100 mL) was added slowly with stirring at 0°C. After completion of the addition of the bromine solution, the reaction mixture was stirred for 5 h. The solid obtained was filtered and recrystallized from acetone. Yield: 86%; m.p. 168-170°C. IR (KBr, cm^{-1}): 3072 (Ar-H), 1683 (C=O), 1220 (C-F), 574 (C-Br); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 5.63 (d, 1H, H_β , *J* = 11.24), 6.20 (d, 1H_α, H , *J* = 11.24), 7.10 -8.27 (m, 8H, Ar-H); Analytical data: Found (Cald): C %: 44.57 (44.59); H%: 2.48 (2.49).

Synthesis of 3,5-bis(4-fluorophenyl)-1*H*-pyrazole (3)

A mixture of 2,3-dibromo-1,3-bis(4-fluorophenyl)propan-1-one **2** (4.04g, 0.01 mol) and hydrazine hydrate (0.7 mL, 0.014 mol) in 20 mL ethanol in presence of 3 mL triethylamine was refluxed for 8 hours. The reaction mixture was cooled and poured into ice-cold water. The precipitate was collected by filtration and purified by recrystallization from ethanol. Yield: 62 %; m.p. 210-212°C; IR (KBr, cm^{-1}): 3421 (NH), 3064 (Ar-H), 1602 (C=N), 1224 (C-F); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.15 (s, 1H, Pyrazole-H), 7.23 -7.90 (m, 8H, Ar-H), 13.33 (s, 1H, NH); LCMS: m/z 257.1 (M^++1); Analytical data: Found (Cald): C % : 70.28 (70.31); H% : 3.90 (3.93); N% : 10.89 (10.93).

Synthesis of hydroxy pyrazolines (4a-f)

A mixture of 2,3-dibromo-1,3-bis(4-fluorophenyl)propan-1-one, **1** (4.04g, 0.01 mol), 4-substituted benzohydrazide (0.012 mol) and triethyl amine (3 mL) was heated under reflux in absolute ethanol (15 mL) for 8 h. The reaction mixture was cooled and poured into ice cold water. The solid separated was filtered, dried and recrystallized from ethanol.

[3,5-Bis(4-fluorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl](4-fluorophenyl)methanone **(4a)**

Yield: 54%; m.p. 122-124°C; IR (KBr, cm^{-1}): 3396 (OH), 2927 (CH_2), 1685 (C=O), 1224 (C-F); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 3.66 (two doublets, 2H, CH_2), 7.62 (s, 1H, OH), 7.11 -7.72 (m, 12 H, Ar-H); LCMS: m/z 396.8 (M^++1); Analytical data: Found (Cald): C % : 66.63 (66.67); H% : 3.79 (3.81); N% : 7.04 (7.07).

[3,5-Bis(4-fluorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl](4-chlorophenyl)methanone **(4b)**

Yield: 59%; m.p. 251-253°C; IR (KBr, cm^{-1}): 3415 (OH), 3062 (Ar-H), 2818 (CH_2), 1629 (C=O), 1593 (C=N), 1234 (C-F); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 3.62 (two doublets, 2H, CH_2), 7.58 (s, 1H, OH), 7.18 -7.84 (m, 12 H, Ar-H); LCMS: m/z 412.9 (M^++1); Analytical data: Found (Cald): C % : 63.98 (64.01); H% : 3.68 (3.66); N% : 6.77 (6.79).

[3,5-Bis(4-fluorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl](4-bromophenyl)methanone **(4c)**

Yield: 52%; m.p. 203-206°C; IR (KBr, cm^{-1}): 3431 (OH), 3076 (Ar-H), 2926 (CH_2), 1680 (C=O), 1598 (C=N), 1236 (C-F); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 3.58 (two doublets, 2H, CH_2), 7.39 (s, 1H, OH), 7.12 -7.76 (m, 12 H, Ar-H); LCMS: m/z 457.9 (M^++2); Analytical data: Found (Cald): C % : 57.75 (57.79); H% : 3.30 (3.31); N% : 6.11 (6.13).

[3,5-Bis(4-fluorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl](4-methylphenyl)methanone **(4d)**

Yield: 46%; m.p. 190-192°C; IR (KBr, cm^{-1}): 3462 (OH), 3021 (Ar-H), 2956 (CH), 1633 (C=O), 1604 (C=N), 1222 (C-F); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 0.96 (s, 3H, CH_3), 3.62 (two doublets, 2H, CH_2), 7.41 (s, 1H, OH), 7.24 -7.88 (m, 12 H, Ar-H); LCMS: m/z 392.9 (M^++1); Analytical data: Found (Cald): C % : 70.37 (70.40); H% : 4.60 (4.62); N% : 7.11 (7.14).

[3,5-Bis(4-fluorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl](biphenyl-4-yl)methanone **(4e)**
Yield: 61%; m.p. 263-265°C; IR (KBr, cm^{-1}): 3232 (OH), 3030 (Ar-H), 2850 (CH), 1683 (C=O), 1604 (C=N), 1270 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.65 (two doublets, 2H, CH_2), 7.05 (s, 1H, OH), 6.92 -7.98 (m, 17 H, Ar-H); LCMS: m/z 454.9 (M^++1); Analytical data: Found (Cald): C % : 73.97 (74.00); H% : 4.42 (4.44); N% : 6.13 (6.16).

[3,5-Bis(4-fluorophenyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl](4-methoxyphenyl)methanone **(4f)**
Yield: 65%; m.p. 141-143°C; IR (KBr, cm^{-1}): 3398 (OH), 3072 (Ar-H), 2935 (CH_2), 1631 (C=O), 1602 (C=N), 1222 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.82 (s, 3H, OCH_3), 3.89 (two doublets, 2H, CH_2), 7.28 (s, 1H, OH), 6.94 -8.05 (m, 12 H, Ar-H); LCMS: m/z 408.9 (M^++1); Analytical data: Found (Cald): C % : 67.61 (67.64); H% : 4.46 (4.44); N% : 6.84 (6.86).

2.2. Biological evaluation

2.2.1. Antimicrobial activity

Antibacterial and antifungal assay were carried out by disc diffusion method. The *in vitro* antimicrobial activity was carried out against 24 h culture of four bacterial strains Gram positive (*Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633), Gram negative (*Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 27853). Bacterial strains used in this study were obtained from National chemical laboratory, Pune, India. Two of the fungal strains viz. *Aspergillus niger* MTCC No. 1344, *Candida albicans* MTCC No. 227 were obtained from IMTECH, Chandigarh, India. The compounds were tested at 0.25mg/disc concentration against both bacterial and fungal strains. DMSO was used as a vehicle. Sterile paper discs (6 mm in diameter) impregnated with tested compound was placed on the surface of the medium and incubated at 37°C for 24 hrs. Antibacterial activity was recorded by measuring the diameter of zone of inhibition. Streptomycin was used as positive reference standard. The entire test was performed in triplicate. The antifungal activity was assayed by inoculating the fungal spores on the potato dextrose agar (PDA) medium pre-impregnated with discs containing tested compound. Nystatin was used as positive reference standard against fungal strains. The results are recorded in Table **1**.

Table 1. Anti-microbial activity of synthesized compounds

	Zone of inhibition in (mm)					
Compound (0.25 mg/disc)	***Staphylococcus aureus***	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Aspergillus niger***	***Candida albicans***
	Antibacterial strains				**Antifungal Strains**	
2	4.2	3.8	-	-	-	-
3	4.0	3.6	-	--	2.8	3.0
4a	5.0	3.5	4.1	3.6	-	-
4b	5.2	4.2	3.9	3.2	4.3	2.5
4c	5.0	3.2	-	-	-	-
4d	2.8	-	-	2.9	-	-
4e	2.0	-	-	3.5	-	-
4f	3.5	3.8	3.1	3.2	2.7	2.9
Control	0	0	0	0	0	0
Streptomycin (10µg/ml)	23	21.9	16.1	19.5	-	-
Nystatin (10µg/ml)	-	-	-	-	19.9	18.2

2.2.2. DPPH radical scavenging assay

The DPPH assay was based on the reported method [22]. Briefly, the DMSO sample of compounds at 100 µg/mL and it was diluted to 4 mL using methanol. To this 1mL of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution in methanol was added. The mixed solution was incubated at room temperature for 30 min. The absorbance of stable DPPH was read at 517 nm using UV-Visible spectrophotometer and the remaining DPPH was calculated. Ascorbic acid was taken as standard. The free radical scavenging activity was expressed as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{[Ac-As]}{[Ac-Ab]} \text{ X } 100$$

Where *Ac* was the absorbance of the control, *As* for the sample and *Ab* for the blank (MeOH+DMSO). Each sample was assayed at 100 µg/mL and all experiments were carried out in triplicate and the % RSC is shown in Table **2.**

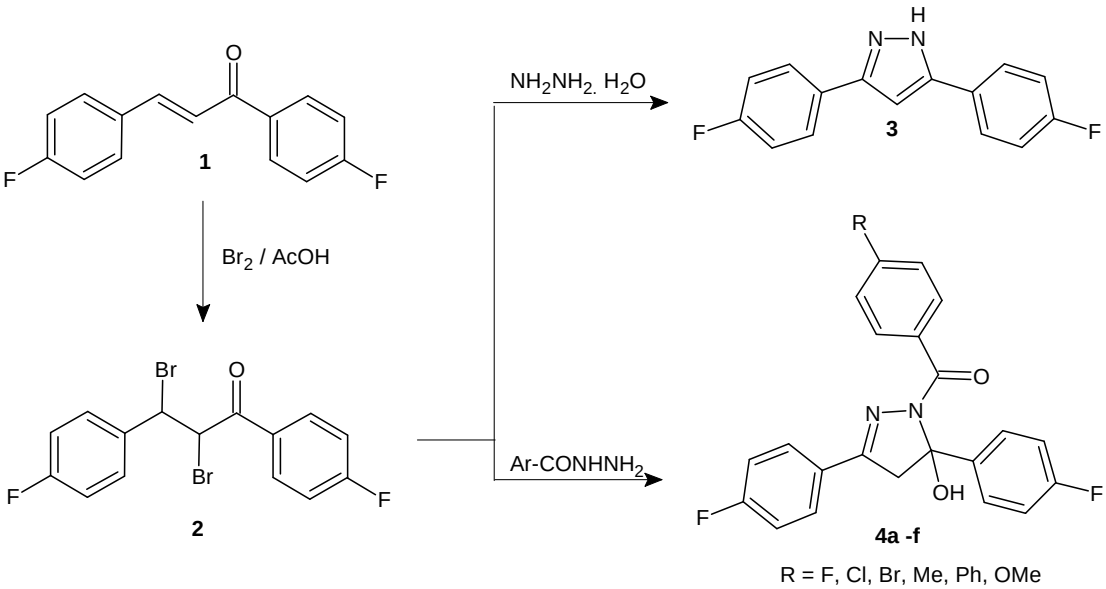
Table 2. DPPH Radical scavenging assay of synthesized compounds

Compounds (100 µg/ml)	% DPPH Scavenging
2	20.46±0.12
3	67.85 ±0.84
4a	37.30±0.54
4b	26.53±0,71
4c	49.29±0.43
4d	16.14 ±0.41
4e	27.14±0.38
4f	46.56±0.31
Ascorbic acid	96.31±0.34

RESULTS AND DISCUSSION

3.1. Chemistry

The synthesis of pyrazole derivatives were carried out by reacting the *α,β*-dibromo 4,4'-difluoro chalcone with various hydrazine derivatives according to the reaction sequence depicted in **Scheme 1**.



Scheme 1. Synthesis of pyrazole derivatives

The stereospecific addition of bromine to carbon-carbon double bond of chalcones was well documented [23, 24]. Bromination of 4,4'-difluoro chalcone **1** with bromine in acetic acid at low temperature resulted in selective formation of the *α,β*-dibromo chalcone derivative **2** which is the the key intermediate in the present study. In the ^{1}H NMR spectrum of *α,β*-dibromo chalcone **2,** *α*- and *β*- protons resonated at δ 6.20 and 5.63 ppm respectively as doublets, which is distinctly different from the resonance frequency of *α*- and *β*- protons of the parent chalcone. The coupling constant *J* = 11.24 Hz indicated a *trans* diaxial orientation of these protons according to Karplus relationship (i.e., α= 180º), which confirmed the expected *anti* addition of bromine to the vinylic moiety of the chalcone. Further multiplets observed in the region δ 7.10-8.27 ppm integrating eight protons were due to protons of aromatic ring.

Reaction of dibromo chalcone **2** with hydrazine hydrate in presence of triethyl amine afforded pyrazole derivative **3**. The IR spectrum of compound **3** showed absorption bands at 3421 cm^{-1} due to NH group. The singlet signal integrating one proton seen at δ 13.33 ppm in the ^1H NMR spectrum of pyrazole derivative **3** is attributed to the NH proton of pyrazole. Further a singlet is observed due to the CH proton of pyrazole ring at δ 7.15 ppm integrating one proton. The mass spectrum showed the presence of molecular ion peak at m/z 257.1 (M++1), hence confirming the proposed structure.

Treatment of *α,β*-dibromo chalcone **2** with acid hydrazides in the presence of triethylamine gave hydroxy pyrazolines **4a-f** rather than the similar pyrazole derivative **3** as explained earlier [10]. Formation of hydroxy pyrazolines **4a-f** were confirmed on the basis of elemental analysis, IR, NMR and mass spectral data. The IR spectrum of hydroxy pyrazoline **4f**, as an example, showed a broad absorption band at 3398 cm^{-1} indicating the presence of hydroxyl group in the compound. The amide carbonyl stretching frequency was observed at 1631 cm^{-1}. A proton of hydroxyl group resonated as a singlet at δ 7.28 ppm in the ^1H NMR spectrum of **4f**. The methylene protons of hydroxy pyrazoline ring appeared as two doublets at δ 3.82 ppm. The appearance of two doublets with coupling constant J = 18 Hz clearly revealed the magnetic nonequivalence of the two protons of CH_2 group adjacent to a chiral center. The mass spectrum showed a molecular ion peak at m/z 408.9 which confirmed its molecular weight. Other hydroxy pyrazolines were also well characterized by spectroscopic analyses to prove their assigned structure. Their characterization data are given in Experimental section.

3.2. Biological Evaluation

3.2.1. Antimicrobial studies

All the synthesized compounds are screened for antibacterial activity against Gram positive (*Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633), Gram negative (*Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 27853). The compounds are also tested against two fungal strains *Aspergillus niger* MTCC No. 1344, *Candida albicans* MTCC No. 227 disc diffusion method [25, 26]. Streptomycin and Nystatin are used as standard antibiotics for antibacterial and antifungal respectively. All the tested compounds showed moderate activty against all tested microorganisms.

3.2.2. DPPH Radical Scavenging Assay

A rapid, simple and inexpensive method to measure antioxidant capacity of substances involves the use of the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. Antioxidants tested on DPPH were also found extremely effective in cell systems. This simple test further provides information on the ability of a compound to donate electrons during antioxidant action [27]. The radical scavenging mechanism is based on the transfer of acidic H-atom from the compound to DPPH radical to form DPPH-H.

Among the tested compounds, compound **3** exhibited good radical scavenging capacity with concentration of 100µg/mL in comparison with the standard ascorbic acid. Other compounds showed mode rate activity. The good radical scavenging capacity of compound **3** is due to the presence of acidic NH proton of pyrazole moiety. The variation exhibited in DPPH scavenging capacity could be attributed to the effect of different substitutions present in the compounds.

CONCLUSION

Pyrazole derivatives were prepared by condensing *α,β*-dibromo 4,4'-difluoro chalcone with various hydrazine derivatives like hydrazine hydrate and substituted acid hydrazides. All these derivatives were characterized by NMR, IR and mass spectral data. All the synthesized products were screened for their *in vitro* antimicrobial and antioxidant properties. Some of the tested compounds exhibited significant DPPH scavenging activity. Hence this study has widened the scope of developing easy method to synthesize pyrazole derivatives as promising antioxidant agents.

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REFERENCES

[1] M.Amir, H. Kumar, S.A.Khan, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 918-922.
[2] R.V.Hes, K.Wellinga, A.C.Grosscurt, *J. Agric. Food Chem.* **1978**, *26*, 915-918.
[3] A.C.Grosscurt, R.V. Hes, K.Wellnga, *J. Agric. Food Chem.* **1979,** *27*, 406-409.
[4] B.K.Sarojini,; M.Vidyagayatri, C.G. Darshanraj, B.R.Bharath, H.Manjunatha, *Lett. Drug Des. Disc.* **2010,** *7*, 214-224.
[5] M.Amir, S.Kumar, *Indian J. Chem.* **2005**, *44B*, 2532-2537.
[6] M.Onishi, M.Yamaguchi, S.Kumagae, H.Kawano, Y.Arikawa, *Inorg. Chim. Acta* **2006**, *359*, 990-997.
[7] K.Tebbji, H.Oudda, B.Hammouti, M.Benkaddour, M.El-Kodadi, A.Ramdani, *Colloids Surf. A Physicochem. Eng. Aspects* **2005,** *259*, 143-149.
[8] E.I.Klimova, M.Marcos, T.B.Klimova, A.T.Cecilio, A.T.Ruben, R.R.Lena, *J. Organomet. Chem.* **1999,** *585*, 106-111.
[9] A.A.-H.Abdel-Rahman, A.E.-S.Abdel-Megied, M.A.-M.Hawata, E.R.Kasem, M.T. Shabaan, *Monatshefte fur Chemie* **2007,** *138*, 889-897.
[10] M.S.Karthikeyan, B.S.Holla, N.S. Kumari, *Eur. J. Med. Chem.* **2007**, *42*, 30-36.
[11] S.Samshuddin, B.Narayana, Z.Baktir, M.Akkurt, H.S.Yathirajan, *Der Pharma Chemica* **2011**, *3*, 487-493.
[12] S.Samshuddin, B.Narayana, B.K.Sarojini, *Molbank* **2011**, *2011*, M745.
[13] S.Samshuddin, B.Narayana, D.N.Shetty, R.Raghavendra, *Der Pharma Chemica* **2011,** *3*, 232-240.
[14] S.Samshuddin, R.J.Butcher, M.Akkurt, B.Narayana, H.S.Yathirajan, B.K. Sarojini, *Acta Cryst.* **2011**, *E67*, o1954-o1955.
[15] J.P.Jasinski, C.J.Guild, S.Samshuddin, B.Narayana, H.S.Yathirajan, *Acta Cryst.* **2010,** *E66*, o2018.
[16] R.Betz, T.Gerber, E.Hosten, S.Samshuddin, B.Narayana, B.K.Sarojini, *Acta Cryst*. **2012**, *E68*, o476.
[17] J.P.Jasinski, C.J.Guild, S.Samshuddin, B.Narayana, H.S.Yathirajan, *Acta Cryst*. **2010,** *E66*, o1948-o1949.
[18] H.-K.Fun, M.Hemamalini, S.Samshuddin, B.Narayana, H. S. Yathirajan, *Acta Cryst.* **2010,** *E66*, o582-o583.
[19] H.-K.Fun, M.Hemamalini, S.Samshuddin, B.Narayana, H. S. Yathirajan, *Acta Cryst.* **2010,** *E66*, o864-o865.
[20] Z.Baktir, M.Akkurt, S.Samshuddin, B.Narayana, H. S. Yathirajan, *Acta Cryst*. **2011,** *E67***,** o1262-o1263.
[21] Z.Baktir, M.Akkurt, S.Samshuddin, B.Narayana, H. S. Yathirajan, *Acta Cryst.* **2011,** *E67*, o1292-o1293.
[22] M.S. Blois, *Nature* **1958**, *26*, 1199-1200.
[23] K.Pitchumani, P.Velusamy, S.Sabithamala, C.Srinivasan, *Tetrahedron* **1994**, *50*, 7903-7912.
[24] Y.Budak, M.Ceylan, *Chinese J. Chem.* **2009**, *27*, 1575-1581.
[25] G.Vardar-Unlu, F.Candan, A.Sokmen, D.Daferera, M.Polissiou, M.Sokmen, *J. Agr. Food Chem.* **2003,** *51*, 63–67.
[26] National Committee for Clinical Laboratory Standards (NCCLS). *Performance standard for antimicrobial disk susceptibility test*. 7th ed. Approved standard NCCLS document M2-A7. Wayne PA .2000.
[27] A.K. Tiwari, *Curr. Sci.* **2004,** *86*, 1092-1100.