Indian Journal of Chemistry
Vol. 48B, April 2009, pp. 563-568

Synthesis and antibacterial activity of 1,3-diaryl-4-cyanopyrazoles

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Received 17 June 2008; accepted (revised) 9 January 2009

A mild, short and simple method for the small scale synthesis of pharmaceutically important 1,3-diaryl-4-cyanopyrazoles **3** is reported from acetophenone arylhydrazones **1** through a two step reaction. All the title compounds have been subjected to *in vitro* antibacterial testing against four pathogenic strains namely, *S.aureus*, *B.subtilis* (gram positive) and gram negative bacteria namely, *P.aeruginosa* and *E.coli*. Preliminary results indicate that most of them exhibited promising activity. Two compounds, namely 1-(4-methylphenyl)-3-(4-bromophenyl)-4-cyanopyrazole **3d** and 1-(4-chlorophenyl)-3-phenyl-4-cyanopyrazole **3e** from this series are found to be equipotent or more potent than the commercial antibiotics (Linezolid and Ciprofloxacin).

Keywords: Vilsmeier-Haack reaction, aldoximes, 4-cyanopyrazoles, antibacterial activity

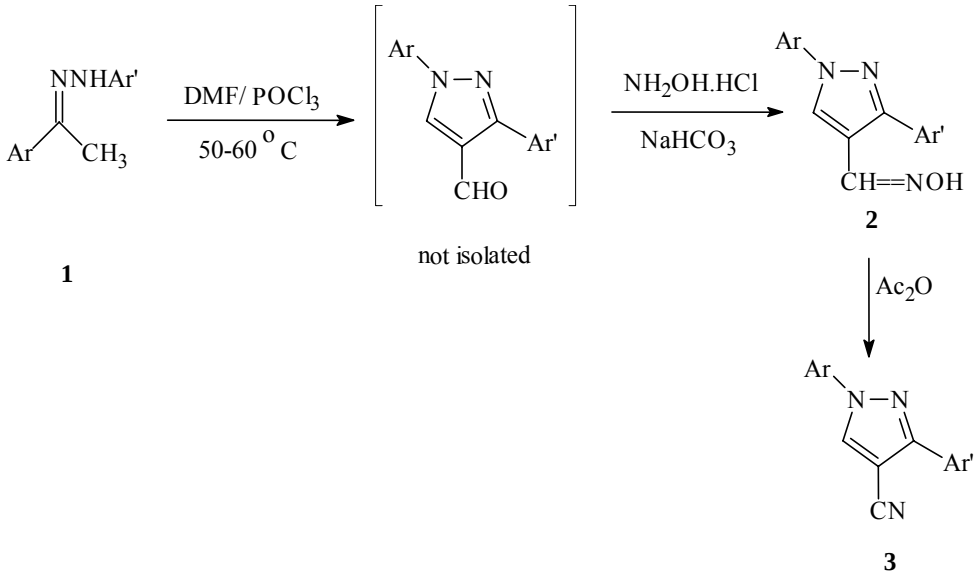
Pyrazoles have attracted a great deal of attention from synthetic community due to their diverse types of biological properties such as antidiabetic[1], anti-bacterial[2], antimicrobial[3], herbicidal[4] and as active pharmacophore in celecoxib[5] (as COX-2 inhibitor) and slidenafil citrate[6] (as phospho-diesterase inhibitor). In the search for the broad spectrum antibacterial agents, it seems to be interesting to develop mild, efficient and convenient synthesis of 4-cyanopyrazoles. There has been particular interest in the synthesis of 4-cyanopyrazoles for two reasons: (i) cyano compounds can be readily converted into other interesting pyrazole derivatives containing various functionalities/heterocyclic moieties[7], (ii) the presence of cyano moiety particularly at the 4 position of pyrazole ring generates compounds with interesting antibacterial activity *in vitro* and *in vivo*[8]. In view of these observations, it was envisaged in the present investigation to undertake the synthesis and evaluation of the antibacterial activity of 1,3-diaryl-4-cyanopyrazoles with an aim to find new and more potent antibacterial agents.

The title compounds were synthesized through the reaction sequence shown in **Scheme I**. The key intermediates, 1,3-diaryl-4-carboxaldehyde oximes **2** were prepared by Vilsmeier-Haack reaction on acetophenone arylhydrazones **1** (generating 1,3-diaryl-4-formylpyrazoles *in situ*) followed by modified work-up with $NH_2OH.HCl$ and $NaHCO_3$ in one-pot[9]. The aldoximes **2** were conveniently converted to the title compounds **3** in good yields by refluxing with Ac_2O. In a recent report, some of these 4-cyanopyrazoles have been obtained by dehydration of the aldoximes using dimethylforamide-thionylchloride complex. Although the results of the present study are comparable with that of the reagent DMF-$SOCl_2$, the Ac_2O mediated route involving simple procedures is advantageous as the additional step of preparing reagent is avoided.

The structural assignments of the new compounds were based on their elemental analyses and spectral data (IR, ^{1}H NMR and MS). The structures of known compounds were confirmed by the comparison of their melting points with literature. IR spectrum showed characteristic peak at ~2225 cm^{-1} for cyano group. ^{1}H NMR showed a singlet around δ 8.2-8.5 for pyrazolyl proton at the 5-position apart from other aromatic protons. The physical characterization data of all the compounds are summarized in **Table I**.

Results and Discussion

All the 4-oximino and 4-cyanopyrazoles synthesized in the present study were tested for antibacterial activity against two gram positive bacteria namely, *Bacillus subtilis*, *Staphylcoccus aureus* and two gram negative bacteria namely, *Escherichia coli* and *Psedumonas aeruginosa*. The compound **3d** showed excellent *in vitro* activity



1

not isolated

2

3

Scheme I

Table I — Physical data and yields of aldoximes **2** and 4-cyanopyrazoles **3**

Compd	Ar	Ar′	m.p. (°C) Obsd	Lit m.p. (°C)	Yield[a] (%)
2a	C_6H_5	C_6H_5	166-67	168-169[9]	82
2b	C_6H_5	4-$NO_2C_6H_4$	97	98-99[9]	79
2c	4-$CH_3C_6H_4$	C_6H_5	140-41	142[9]	80
2d	4-$CH_3C_6H_4$	4-BrC_6H_4	176-180	-	76
2e	4-ClC_6H_4	C_6H_5	167-168	169[7]	74
2f	4-ClC_6H_4	4-BrC_6H_4	208-210	-	80
2g	4-$NO_2C_6H_4$	C_6H_5	195-96	-	60
2h	4-$NO_2C_6H_4$	4-$OCH_3C_6H_4$	195-96	-	62
3a	C_6H_5	C_6H_5	110-111	-	65
3b	C_6H_5	4-$NO_2C_6H_4$	120-122	-	69
3c	4-$CH_3C_6H_4$	C_6H_5	123	124[7]	62
3d	4-$CH_3C_6H_4$	4-BrC_6H_4	140-142	-	64
3e	4-ClC_6H_4	C_6H_5	154-155	156[7]	65
3f	4-ClC_6H_4	4-BrC_6H_4	180-81	-	78
3g	4-$NO_2C_6H_4$	C_6H_5	208-210	-	76
3h	4-$NO_2C_6H_4$	4-$OCH_3C_6H_4$	205-207	-	73

[a] Yields of the isolated products **2** and **3** starting from **1**

against both gram positive bacteria *i.e*. MIC 2 μg/mL and displayed moderate inhibition against gram negative bacteria at MIC 8 μg/mL in DMSO. Excellent activity against *S. aureus* at 2 μg/mL was also shown by **3e** followed by good inhibition against *B. subtilis* at 4 μg/mL and exhibited moderate activity (MIC 8 μg/mL) against gram negative bacteria. The potency of **3a** was found to be less against both gram positive and gram negative bacteria. The compound **3b** showed strong activity against *S. aureus*, *B. subtilis* and *E. coli* (MIC of 4 μg/mL) and modest activity (MIC 8 μg/mL) against *P. aeruginosa*. The compounds **3c** and **3f** displayed similar inhibitory action against all the test organisms. They possessed good activity against *S.aureus*, *B.subtilis* and *E.coli* showing MIC of 4 μg/mL and inhibited the growth of *P. aeruginosa* at 8 μg/mL. The compounds **3g,h** were found to be more potent against gram negative

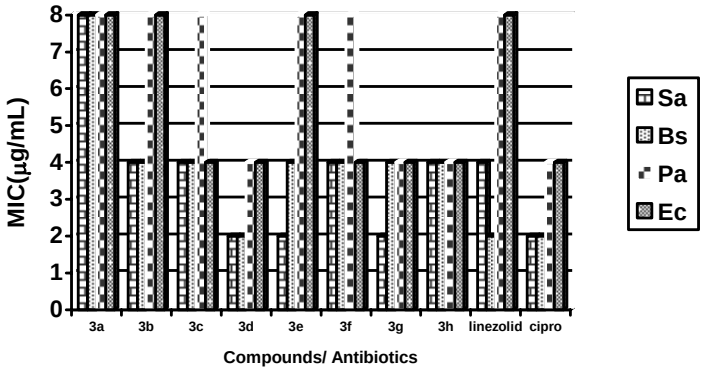


Figure 1 — Comparison of MIC of test compounds with commercial antibiotics up to MIC 8 (µg/mL)

Table II — *In vitro* antibacterial spectrum of aldoximes **2a-h** and 4-cyanopyrazoles **3a-h** by using agar diffusion assay

Compd	Diameter of inhibition zone in mm[b]			
	S.aureus	*B.subtilis*	*P.aeruginosa*	*E.coli*
2a	13.66±0.577	10.66±0.577	-	-
2b	11.66±0.577	11.00±1.00	-	-
2c	11.33±1.154	11.66±0.577	-	-
2d	13.66±1.154	11.33±0.577	-	-
2e	11.33±0.577	9.66±0.577	-	-
2f	12.33±1.527	11.66±0.577	-	-
2g	10.33±1.527	10.66±0.577	-	-
2h	9.66±0.577	10.66±0.577	-	-
3a	21.66±0.577	23.66±0.577	22.6±0.577	19.66±0.577
3b	22.33±0.577	22.0±0.00	23.6±0.577	23.66±0.577
3c	26.66±0.707	25.66±0.577	20.6±1.527	19.3±0.577
3d	32.66±0.577	30.33±1.154	21.3±1.414	21.6±0.577
3e	31.0±1.00	25.66±1.154	22.0±1.0	22.00±0.00
3f	26.66±0.707	26.33±0.707	20.6±0.577	22.33±0.577
3g	27.66±0.577	25.66±0.577	24.6±0.577	23.33±0.577
3h	26.66±0.577	28.66±0.707	20.6±0.577	24.66±1.154
DMSO	9.33±.577	8.66±1.154	7.66±0.577	8.00±0.00
Linezolid	24.66±0.577	15.66±0.707	14.66±0.577	15.83±0.707
Ciprofloxacin	32.66±0.577	29.33±0.577	28.0±0.0	28.66±0.577

[b]Mean of three replicates; ±standard deviation

-, no activity

Sa-*Staphylcoccus aureus* (MTCC 3160), Bs-*Bacillus subtilis* (MTCC 121), Ec – *Escherichia Coli* (MTCC 51) and Pa – *Psedumonas aeruginosa* (MTCC 741)

bacteria (MIC 4 µg/mL) and showed good inhibition against gram positive bacteria (MIC 4 µg/mL). Aldoximes **2a-h** were virtually inactive against gram negative bacteria, but have modest to poor activity (MIC 16-64 µg/mL) against gram positive bacteria. A careful investigation of MIC data of 4-cyanopyrazoles **3a-h** showed very potent activity against both gram positive and gram-negative organisms **(Figure 1)**. Additional enhancement in antibacterial activity of cyano analogues **3a-h** can be attributed to the presence of pharmacologically active CH_3, Cl, Br and NO_2 groups. The presence of NO_2 is sufficient to

Table III — Minimum inhibitory concentration (MIC) of **2a-h** and **3a-h** against test bacteria by using microplate dilution method

Compd	MIC (μg/mL)			
	Sa	Bs	Pa	Ec
2a	>64	>32	>64	64
2b	64	32	>64	>64
2c	32	32	>64	>64
2d	32	16	>64	32
2e	16	16	64	32
2f	32	16	64	32
2g	64	64	>64	>64
2h	>64	32	>64	>64
3a	8	8	8	8
3b	4	4	8	8
3c	4	4	8	4
3d	2	2	4	4
3e	2	4	8	8
3f	4	4	8	4
3g	2	4	4	4
3h	4	4	4	4
Linezolid	4	2	8	8
Ciprofloxacin	2	2	4	4

Sa-*Staphylcoccus aureus* (MTCC 3160), Bs-*Bacillus subtilis* (MTCC 121), Ec – *Escherichia coli* (MTCC 51) and Pa – *Psedumonas aeruginosa* (MTCC 741)

enhance the activity against bacteria irrespective of its position within the molecule.

With an aim to find new and more potent antibacterial agents, especially when one considers the gram negative bacteria, the present study has led to the discovery of 4-cyanopyrazoles *via* a mild, efficient and convenient route. The pyrazole ring plays a subtle, yet important role. The presence of cyano moiety on pyrazole ring seems to be significant for contributing to antibacterial activity as the corresponding 4-oximinopyrazoles did not show any significant activity **(Tables II** and **III)**. The compounds **3a-h** exhibited excellent antibacterial activity against test bacteria namely *S.aureus* (MTCC 3160) and *B.subtilis* (MTCC 121) that were gram positive and *E.coli* and *P.aeruginosa* which were gram negative. Thus, these compounds represent promising new leads towards combating the emerging drug-resistant pathogens. Efforts are in progress to test these compounds against drug resistance pathogens and their evaluation in human system for their toxicity.

Experimental Section

Melting points were recorded in open capillaries in an electrical apparatus and are uncorrected. ^{1}H NMR was recorded on a Bruker 300 MHz instrument using TMS as internal standard. IR spectra were recorded on a Perkin-Elmer 1800 FT-IR spectrometer.

General procedure for preparation of 1,3-diaryl-pyrazole-4-aldoximes, 2a-h

To a cold solution of DMF (10 mL) and phospho-rousoxy chloride (0.5 mL, 6 mmole), was added appropriate acetophenone arylhydrazone (**1**, 4 mmole). The mixture was stirred at 50-60°C for 5-6 hr, cooled to RT and then poured into ice cold solution of hydroxylamine hydrochloride followed by sodium bicarbonate. The reaction mixture was stirred overnight. The solid product thus obtained was filtered, washed with water and purified by recrystallization from ethanol to give 1,3-diarylpyrazole-4-aldoxime **2**.

Characterization data for 1,3-diarylpyrazole-4-carboxaldehyde oximes, 2a-h

2a: m.p. 166-67 °C (lit.[9] 168-69°C).

2b: m.p. 97°C (lit.[9] 98-99°C).

2c: m.p. 140-41°C (lit.[9]142°C).

2d: IR (KBr): 3250 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.42 (s, 3H, CH_3) 7.2-7.7 (m, 9H, Ar-H), 8.27 (s, 0.5H), 8.35 (s, 0.5H), 8.95 (s, 1H, C_5-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.3 (CH_3), 107 (C_4, pyrazole ring), 115.6-136 (aromatic carbons), 133 (C_5, pyrazole ring), 145.8 (CH=), 146.98 (CH=), 150.3 (C_3, pyrazole ring). Anal. Found: C, 57.46; H, 4.0; N, 11.82. $C_{17}H_{14}N_3OBr$ requires C, 57.46; H, 3.94; N, 11.83%.

2e: m.p. 167-68°C (lit.[7] 169°C).

2f: IR (KBr): 3255 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.2-7.7 (m, 9H, Ar-H), 8.27 (s, 0.5H), 8.35 (s, 0.5H), 8.95 (s, 1H, C_5-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 107 (C_4, pyrazole ring), 115.6-136 (aromatic carbons), 133.19 (C_5, pyrazole ring), 145.4 (CH=), 146.49 (CH=), 151.3 (C_3, pyrazole ring). Anal. Found: C, 51.4; H, 3.04; N, 11.4. $C_{16}H_{11}N_3OClBr$ requires C, 51.2; H, 2.93; N, 11.2%.

2g: IR (KBr): 3235 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.4-8.0 (m, 9H, Ar-H), 8.27 (s, 0.5H), 8.35 (s, 0.5H), 8.95 (s, 1H, C_5-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 107 (C_4, pyrazole ring), 115.6-140.9 (aromatic carbons), 133 (C_5, pyrazole ring), 145.8 (CH=), 146.98 (CH=), 153.9 (C_3, pyrazole ring).

Anal. Found: C, 62.50; H, 3.92; N, 18.2. $C_{16}H_{12}N_4O_3$ requires C, 62.33; H, 3.89; N, 18.18%.

2h: IR (KBr): 3255 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.6 (s, 3H, OMe), 7.4-8.0 (m, 9H, Ar-H), 8.25 (s, 0.5H), 8.35 (s, 0.5H), 8.95 (s, 1H, C_5-H). Anal. Found: C, 60.45; H, 4.11; N, 16.45. $C_{17}H_{14}N_4O_4$ requires C, 60.35; H, 4.14; N, 16.5%.

General procedure for preparation of 1,3-diaryl-4-cyanopyrazoles, 3a-h

A mixture of aldoximes (**2,** 1 mmole) in acetic anhydride (2 mL) was refluxed for 30 min. The reaction mixture was quenched by pouring into crushed ice with stirring and neutralized with sodium bicarbonate. The solid which separated after standing overnight was filtered, washed with water, dried and recrystallised from ethanol to afford the pure cyanopyrazoles **3**.

Characterization data for 1, 3-diaryl-4-cyanopyrazoles, 3a-h

3a: IR (KBr): 2224 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.2-7.8 (m, 10 H, Ar-H), 8.47 (s, 1H, C_5-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 91.8 (C_4, pyrazole ring), 114.2 (CN), 115.6-136 (aromatic carbons), 133 (C_5, pyrazole ring), 153.9 (C_3, pyrazole ring). Anal. Found: C, 78.3; H, 4.58; N, 16.98. $C_{16}H_{11}N_3$ requires: C, 78.32; H, 4.48; N, 17.14%. MS: *m/z* M^+ 245.

3b: IR (KBr): 2230 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.4-8.2 (m, 9H, Ar-H), 8.47 (s, 1H, C_5-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 91.28 (C_4, pyrazole ring), 113.4 (CN), 115.6-136 (aromatic carbons), 133 (C_5, pyrazole ring), 151.9 (C_3, pyrazole ring). Anal. Found: C, 66.2; H, 3.40; N, 19.30. $C_{16}H_{10}N_4O_2$ requires: C, 66.26; H, 3.44; N, 19.31%.

3c: m.p. 123°C (lit.[7] 124°C).

3d: IR (KBr): 2225 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 2.4 (s, 3H, CH_3), 7.4-7.9 (m, 9H, Ar-H), 8.48 (s, 1H, C_5-H). Anal. Found: C, 60.09; H, 3.62; N, 12.40. $C_{17}H_{12}N_3Br$ requires: C, 60.1; H, 3.66; N, 12.42%.

3e: m.p. 154-55°C (lit.[7] 156°C).

3f: IR (KBr): 2225 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.4-8.1 (m, 9H, Ar-H), 8.34 (s, 1H, C_5-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 91.28 (C_4, pyrazole ring), 114.4 (CN), 115.6-136 (aromatic carbons), 133 (C_5, pyrazole ring), 153.9 (C_3, pyrazole ring). Anal. Found: C, 53.32; H, 2.58; N, 11.60. $C_{16}H_9N_3ClBr$ requires C, 53.18; H, 2.50; N, 11.69%. MS: *m/z* M^+ 361.

3g: IR (KBr): 2225 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.4-8.1 (m, 9H, Ar-H), 8.52(s, 1H C_5-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 91.28 (C_4, pyrazole ring), 113.4 (CN), 115.6-146 (aromatic carbons), 133 (C_5, pyrazole ring), 151.9 (C_3, pyrazole ring). Anal. Found: C, 66.3; H, 3.40; N, 19.2. $C_{16}H_{10}N_4O_2$ requires C, 66.2; H, 3.44; N, 19.3%.

3h: IR (KBr): 2225 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.8 (s, 3H, OCH_3) 7.4-8.1 (m, 9H, Ar-H), 8.52 (s, 1H, C_5-H). Anal. Found: C, 63.65; H, 3.8; N, 17.45. $C_{17}H_{12}N_4O_3$ requires C, 63.75; H, 2.50; N, 17.5%.

Biological assays

Medium

Solid medium used for the study were Muller Hinton agar (MHA) of the following composition: beef infusion 300 gL^{-1}, casein acid hydrolysate 17.5 gL^{-1}, agar-agar 17 gL^{-1} and sterilled distilled water 1000 mL, adjusted to *p*H 7.4.

Microorganisms

Microorganisms used were *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 51) and *Psedumonas aeruginosa* (MTCC 741). All of them were obtained from the microbial type culture collection (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, India.

Primary screening

The antibacterial activity of the newly synthesized compounds was evaluated by agar-well diffusion method[10]. The bacterial cultures were maintained on Muller-Hinton agar by subculturing them on fresh slants after every four weeks and incubating them at the respective temperature for 24 hr. All stock cultures were stored under refrigeration at 4°C. For the evaluation of antimicrobial activity, 24 hr fresh culture of bacteria was suspended in sterile distilled water to obtain a turbidity of 0.5 McFarland units. The final inoculum size was adjusted to 5×10^5 CFU/mL.

Twenty milliliters of agar media was poured into each Petri dish and plates were swabbed with the broth culture of the respective microorganisms and kept for 15 min for absorption to take place. Using a punch, 8 mm diameter wells were bored in the seeded agar plates and a 50 mL portion of 2.5 µg/mL

concentration of each compound reconstituted in DMSO was added into the well. After holding the plates at RT for 2 hr to allow diffusion of the compounds into the agar, the plates were incubated at 37°C for 24 hr. The diameter of zone of growth inhibition around each well was measured after incubation using a vernier caliper (**Table II**).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) values of the pure compounds were further tested to determine the concentration at which they were bacteriostatic and bactericidal using microplate dilution method[11,12] against two gram positive (*B. subtilis* and *S.aureus*) and two gram negative (*E.coli* and *P.aeruginosa*) bacteria. In order to test the concentration from 64-0.12 μg/mL three sterile 96-well microplate with lid (Tarson, U bottom well) was set up as follows: in wells in row A were placed 200 μL portions of each compound in sterile MHB; wells in row B to H received 100 μl of sterile MHB. Serial two fold dilution were carried out from row A to row H and excess broth (100 μL) was discarded from row H to each well was added 100 μL of incoulum. The inoculum was prepared using a 16 hr culture adjusted by reference to the 0.5 McFarland standard (10^8 cells/mL) and further diluted with MHB to achieve approximately 10^6 CFU/mL a positive control (containing inoculum but no compound) and negative control (containing compound but no inoculum) were included on each microtitre plate. Ciprofloxacin (Himedia, Batch no. SD 080) and Linezolid (Alembic, Batch no. 6893002;) were used as standard drugs. The contents of the wells were mixed and incubated for 24 to 48 hr at 37°C.

The minimum inhibitory concentration (MIC) was the lowest concentration of extract that completely inhibited the growth of organism in the micro dilution wells, as detected by the unaided eye (**Table III**).

Acknowledgements

The authors are thankful to CSIR, New Delhi for the award of Junior Research Fellowship to Pooja Ranjan and DRDO (ERIP/ER/0103294/M/01), New Delhi for the award of Senior Research Fellowship to Kamaljeet Pannu to carry out this work.

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