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Synthesis, characterization and anti-microbial screening of novel heterocyclic system containing bridgehead nitrogen atom.

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ABSTRACT

The nicotino hydrazide (1) on reaction with carbon disulphide and ethanolic potassium hydroxide followed by treatment with hydrazine hydrate give 4-amino-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiol (3). The compound (3) when treated carbon disulphide in ethanolic potassium hydroxide undergo cycloaddition to produce 3-(pyridin-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazolo-6(5*H*)-thione(4). Similar type of cycloaddition occurs on stirring (3) with acetyl chloride to produce 6-methyl-3-(pyridin-3-yl)-[1,2,4] triazole [3,4-*b*][1,3,4] thiadiazole (5). 3-(pyridin-3-yl)-[1,2,4] triazolo [3,4-*b*][1,3,4] thiadiazolo-6(5*H*)-one (6), 6-phenyl-3-(pyridin-3-yl)-7*H*-[1,2,4] triazolo [3,4-*b*][1,3,4] thiadiazine (7), 3-(pyridin-3-yl)-7*H*-[1,2,4] triazolo [3,4-*b*][1,3,4] thiadiazine(8), 3-(pyridin-3-yl)-5*H*-[1,2,4] triazolo [3,4-*b*][1,3,4] thiadiazin-6(7*H*)-one(9), 6,7-diphenyl-3-(pyridin-3-yl)-5*H*-[1,2,4] triazolo[3,4-*b*][1,3,4] thiadiazine(10), 6-phenyl-3-(pyridin-3-yl)-[1,2,4] triazolo [3,4-*b*][1,3,4] thiadiazole (11), 3-(pyridin-3-yl)-5,6-dihydro-[1,2,4] triazolo[3,4-*b*][1,3,4] thiadiazin-7-one(12) and N-phenyl-3-(pyridin-3-yl)-[1,2,4] triazolo[3,4-*b*][1,3,4] thiadiazol-6-amine(13) are obtained in good yield on reacting (3) with urea, phenacyl bromide, chloro acetaldehyde, benzoin, benzoic acid, chloro acetyl chloride and isothio cyanate respectively. The newly synthesized compounds have been characterized by elemental analysis and spectral studies. The synthesized compounds have been evaluated for antimicrobial activity.

Keywords: Nicotino hydrazide, Triazole, Triazolothiadiazines, Triazolothiadiazole and Anti-microbial activity.

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INTRODUCTION

The heterocyclic systems encompassing 1,2,4- triazole, 1,3,4- thiadiazole and thiadiazine are explored to the maximum extent owing to their wide spectrum of pharmacological activities, such as fungicidal, insecticidal, bactericidal, herbicidal [1], anti-tumor [2], anti-inflammatory [3], anti-viral [4] and CNS stimulant properties [5]. Various 1, 2, 4-triazole and N-bridged heterocycles derived from them are found to be associated with divers pharmacological activity [6-11]. The 1, 2, 4-triazole nucleus has recently been incorporated into a wide variety of therapeutically interesting molecules [12]. Prompted by the above observation and in continuation of our research for biologically active bridgehead nitrogen containing heterocycles [13, 14]. It was decided to synthesize 1, 3, 4- thiadiazole and thiadiazine derivatives. All the synthesized compounds have been supported by their spectral data and screened for their antimicrobial activity.

MATERIALS AND METHODS

Experimental

Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was ascertained by TLC on silica gel-G plate. Characterizations of synthesized compounds were done by spectral studies. IR spectra were taken in KBr on a THERMONICOLET NEXUS-670 Spectrophotometer. ^{1}H NMR spectra were recorded on AVANCE-300MH$_Z$ Spectrophotometer in DMSO-d_6 with TMS as internal standard. The chemical shift values are in delta (ppm). Physical data and antimicrobial activities of synthesized compounds were recorded in Table-1, 2 & 3.

Preparation of 4 amino-5-(pyridin-3-yl)-4*H*-1, 2, 4-triazole-3-thiol (3)

To a continuously stirred solution of Potassium Hydroxide (8.4 gm, 0.15 mol) and nicotino hydrazide (1) (18.5 gm, 0.1 mol) in absolute ethanol (100 ml), CS_2 (11.2 gm, 0.15 mol) was added drop wise. After the complete addition, the mixture was diluted with absolute ethanol (75 ml) and agitated for 16 hrs. It was then diluted with dry ether (100 ml) and the precipitate solid was collected by filtration, washed with ether and dried under vacuum to obtain crude suspension of potasium dithio carbazinate (2).

To above suspension, Hydrazine hydrate (10 ml, 0.2mol) was added and the mixture was refluxed for about an hour. The colour of the reaction mixture changed to green with the evolution of hydrogen sulphide and a homogeneous mass was obtained. It was then cooled and diluted with cold water (100 ml). The cold mixture was acidified with concentrated HCl. The solid separated was filtered, washed with water, dried and recrystallized from methanol.

IR (KBr):3035.5 cm^{-1} (Pyridine C-H), 1600.1 cm^{-1} (C=N), 2575.4 cm^{-1} (SH), 1260.0 cm^{-1} (NH_2).
^{1}H NMR (DMSO-d_6):7.6-8.8 (m, Pyridine-H), 2.1 (s, 2H, NH_2), 3.1 (s, 1H, SH).

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Table – 1Physical data of Compounds

Compound	M.P (^{o} C)	Yield (%)	Molecular Formula	Elemental Analysis % Found/(Calcd)			
				C	H	N	S
4	85	62	$C_8H_5N_5S_2$	40.85 (40.35)	2.14 (2.20)	29.76 (29.35)	27.25 (29.35)
5	89	60	$C_9H_7N_5S$	49.75 (49.80)	3.25 (3.23)	32.24 (32.15)	14.76 (14.80)
6	80	65	$C_8H_5N_5OS$	43.85 (43.80)	2.30 (2.25)	31.95 (31.84)	14.65 (14.70)
7	91	71	$C_{15}H_{11}N_5S$	61.45 (61.50)	3.80 (3.83)	23.85 (23.87)	10.95 (10.84)
8	72	73	$C_9H_7N_5S$	49.75 (49.81)	3.25 (3.24)	32.25 (32.18)	14.75 (14.78)
9	70	70	$C_9H_7N_5OS$	46.35 (46.40)	3.00 (3.03)	30.00 (30.07)	13.75 (13.79)
10	86	74	$C_{21}H_{15}N_5S$	68.30 (68.32)	4.10 (4.13)	19.00 (18.94)	08.70 (08.65)
11	88	71	$C_{14}H_9N_5S$	60.18 (60.13)	3.24 (3.26)	25.10 (25.11)	11.50 (11.52)
12	71	73	$C_9H_7N_5OS$	46.35 (46.38)	3.00 (3.02)	30.00 (30.05)	13.75 (13.80)
13	89	78	$C_{14}H_{10}N_6S$	57.15 (57.11)	3.40 (3.37)	28.50 (28.52)	10.90 (10.82)

Preparation of 3-(pyridin-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazolo-6(5*H*)-thione(4).

A mixture of (3) (0.01 mol), carbon disulphide (0.01 mol), and dry pyridine (20 ml) was heated under reflux for 3 hr. it was cooled and poured on ice-water. A solid product (4) was obtained by filtration and purification by recrystallization from ethanol.

IR (KBr):3030.6 cm^{-1} (Pyridine C-H), 1601.6 cm^{-1} (C=N), 1375.4 cm^{-1} (C=S), 3250.0 cm^{-1} (Hetero N-H).
^{1}H NMR (DMSO-d_6):7.9-8.9 (m, Pyridine-H), 2.1 (s, 1H, NH-).

Preparation of 6-methyl-3-(pyridin-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole(5).

To a solution of (3) (0.01 mol) in dry pyridine (25 ml), the acetyl chloride were added drop wise. The reaction mixture was stirred at rt for 45 min and then poured into crushed ice. The solid product (5) obtained by filtration were purified by recrystallization from ethanol.

IR (KBr):3031.5 cm^{-1} (Pyridine C-H), 1590.0 cm^{-1} (C=N), 2920.1 cm^{-1} (methyl C-H).
^{1}H NMR (DMSO-d_6):7.5-8.7 (m, Pyridine-H), 2.4 (s, 3H, CH_3-).

Preparation of 3-(pyridin-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazolo-6(5*H*)-one(6).

A mixture of (3) (0.1 mol) and urea (0.13 mol) was heated at 180-900C for 6 hr. the reaction mixture was cooled and added to a solution of sodium hydroxide (5%, 20 ml), then filtered and the filtrate acidified with dilute HCl. The solid product (6) was purified by recrystallized from ethanol.

IR (KBr):3030.9 cm^{-1} (Pyridine C-H), 1600.9 cm^{-1} (C=N), 1640.2 cm^{-1} (amide C=O), 3242.1 cm^{-1} (Hetero N-H).
^{1}H NMR (DMSO-d_6):7.3-8.2 (m, Pyridine-H), 8.1 (s, 1H, NH-).

Preparation of 6-phenyl-3-(pyridin-3-yl)-7*H*-[1, 2, 4] triazolo [3,4-*b*][1,3,4]thiadiazine (7)

A suspension of (3) (0.1 mol) and phenacyl bromide 90.13 mol) in absolute ethanol (25 ml) was heated under reflux for 3 hr, followed by addition of (0.01 mol) anhydrous sodium acetate. The reaction mixture was heated for additional 1 hr, then cooled and poured into ice-cold water. The solid product (7) was purified by recrystallized from ethanol.

IR (KBr):3040.5 cm^{-1} (Pyridine C-H), 1600.9 cm^{-1} (C=N), 3010.5 cm^{-1} (Ar C-H).
^{1}H NMR (DMSO-d_6):7.4-8.8 (m, Pyridine-H), 3.1 (s, 2H, CH_2-), 7.0-7.5 (m, Ar-H)

Preparation of 3-(pyridin-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine(8).

A mixture of (3) (0.01 mol), chloro acetaldehyde (0.02 mol) and Conc. Hcl (2ml) in ethanol (50 ml) was refluxed for 3 hr. after removal of ethanol under reduced pressure, the resulting solid was filtered and washed with water. The crude product was purified by recrystallized from ethanol.

IR (KBr):3030.0 cm^{-1} (Pyridine C-H), 1590.1 cm^{-1} (C=N).
^{1}H NMR (DMSO-d_6):7.9-8.6 (m, Pyridine-H), 3.8 (s, 2H, CH_2-), 7.6 (t,1H, =CH)

Preparation of 3-(pyridin-3-yl)-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6(7*H*)-one(9).

A mixture of (3) (2.08 g, 0.01 mol), chloroacetic acid (0.94 g, 0.01 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) in absolute alcohol (50 ml) was heated under reflux for 6 hr and cooled in ice. The solid thus separated was filtered, washed thoroughly with water and crystallized from methanol.

IR (KBr):3035.1 cm^{-1} (Pyridine C-H), 1600.9 cm^{-1} (C=N), 1642.5 cm^{-1} (amide C=O), 3230.1 cm^{-1} (Hetero N-H).
^{1}H NMR (DMSO-d_6):7.4-8.6 (m, Pyridine-H), 3.9 (s, 2H, CH_2-), 8.1(s,1H, NH)

Preparation of 6,7-diphenyl-3-(pyridine-3-yl)-5*H*-[1,2,4] triazolo [3,4-b] [1,3,4] thiadiazine(10)

A mixture of (3) 91.04 g, 0.005 mol) and benzoin (1.06 g, 0.005 mol) in ethanol (50 ml) was heated to get a clear solution and to the hot solution was added 2N KOH (1.0 ml). the resulting mixture was refluxed with constant stirring for about half an hour. The yellow precipitate thus separated out was filtered, washed with water and crystallized from ethanol.

IR (KBr):3042.8 cm^{-1} (Pyridine C-H), 1610.1 cm^{-1} (C=N), 3015.1 cm^{-1} (Ar C-H), 3220.5 cm^{-1} (Hetero N-H)
^{1}H NMR (DMSO-d_6):7.5-8.5 (m, Pyridine-H), 2.3 (s, 1H, NH-), 7.1-7.4 (m, Ar-H)

Preparation of 6-phenyl-3-(pyridin-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (11).

A mixture of (3) (1.04 g, 0.005 mol) and benzoic acid (0.78 g, 0.005 mol) in $POCl_3$ (10 ml) was heated on an oil-bath at 120^{0}C for 1 hr. The reaction mixture was cooled, poured into ice and neutralized with aqueous potassium carbonate solution. The solid thus separated was filtered, washed thoroughly with water and crystallized from ethanol.

IR (KBr):3040.5 cm^{-1} (Pyridine C-H), 1600.5 cm^{-1} (C=N), 3020.5 cm^{-1} (Ar C-H),
^{1}H NMR (DMSO-d_6):7.4-8.5 (m, Pyridine-H), 7.1-7.4 (m, Ar-H).

Preparation of 3-(pyridin-3-yl)-5,6-dihydro-[1,2,4] triazolo [3,4-*b*][1,3,4] thiadiazin-7-one(12).

A mixture of (3) (0.1 mol) and chloroacetyl chloride (0.1 mol) in dry dioxane (30 ml) was allowed to stand at rt overnight. The precipitated solid was filtered off and purified by recrystallized from benzene.

IR (KBr):3035.5 cm^{-1} (Pyridine C-H), 1605.2 cm^{-1} (C=N), 1720.2 cm^{-1} (ketonic C=O), 3235.3 cm^{-1} (Hetero N-H).
^{1}H NMR (DMSO-d_6):7.4-8.1 (m, Pyridine-H), 3.9 (d, 2H, CH_2-), 2.2(t,1H, NH).

Preparation of N-phenyl-3-(pyridin-3-yl)-[1,2,4] triazolo [3,4-*b*][1,3,4] thiadiazol-6-amine(13).

A mixture of (3) (0.1 mol), phenyl isothiocyanate (0.1 mol) and powered sodium hydroxide (0.8 g) in DMF (25 ml) was stirred at rt for 24 hr. the reaction mixture was poured into dilute acetic acid (5%, 15 ml). The precipitated product was filtered and purified by recrystallized from ethanol. The thiosemicarbazide derivative was fused in an oil-bath above its melting point. The product was cooled, diluted with ethyl acetate, and filtered. The solid product was purified by recrystallized from ethanol.

IR (KBr):3035.9 cm^{-1} (Pyridine C-H), 1602.8 cm^{-1} (C=N), 3030.1 cm^{-1} (Ar C-H), 3230.1 cm^{-1} (N-H).
^{1}H NMR (DMSO-d_6):7.4-8.4 (m, Pyridine-H), 6.4-7.1 (m, Ar-H), 4.1(s, 1H, NH).

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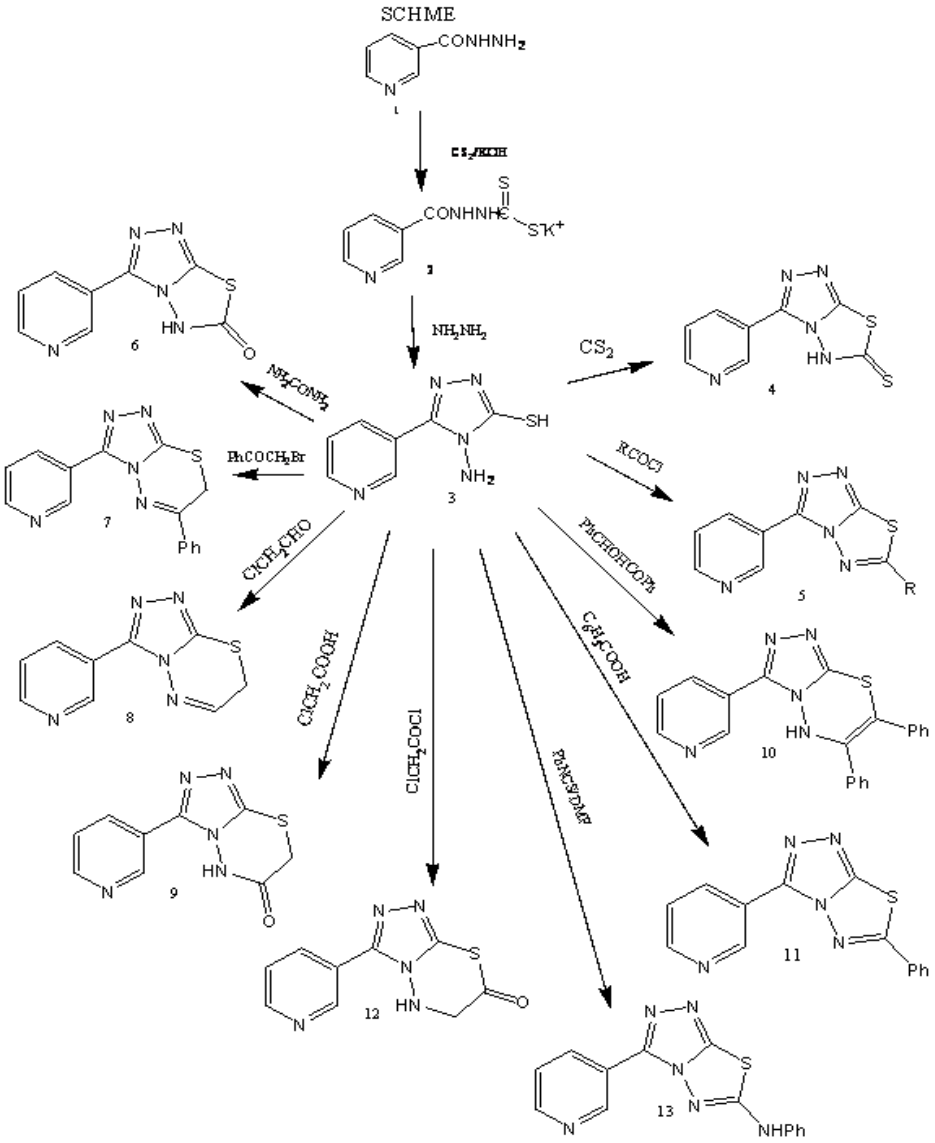

Anti-Microbial activity [15, 16]

The synthesized compounds were reconstituted in dimethyl formamide (DMF) as this does not demonstrate any antibacterial activity by itself. Initially, a suspension of nutrient agar medium is prepared. These suspension medium is then inoculated by 100 µl of *Bacillus cereus* ATCC11778 organism and the inoculation is possible only at 50^0C. Below this temperature the suspension of agar medium gets solidified and hence uniform distribution of test organism cannot be achieved. Then immediately pour the inoculated agar medium into a sterile peteridish under aseptic conditions. Maintenance of aseptic condition is an essential factor, which does not allow the contamination of other microorganism. Now the peteridishes are kept aside for few minutes, in order to get solidified, forming a thin uniform layer of about 2-4 mm.

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sterile discs made up of whatmann paper are used to apply the standard and test solutions on to the culture media. Initially the discs are socked in control, standard drugs (Ampicillin & Ketoconazole) and test solutions separately and then, place on inoculated culture medium aseptically. Fine distance should be kept between these discs. Peteridishes are tightly packed and subjected to incubation at 37^0C to 40^0C for 48 hrs. Bacterial growth inhibition was determined as the diameter of inhibition zones around the disc. All tests were performed in triplicates. The resultant clear zones were measured in millimeters (mms) and compared against standard. Similar procedure carried out by using following organisms *Staphylococcus aureous*ATCC9144, *Micrococcus luteus* ATCC25923, *Staphylococcus epidermidis* ATCC10987, *Klebsiella pneumonia* ATCC29212, *Escherichia coli* ATCC25922, *Candida albicans* ATCC 2091 and *Aspergillus niger* ATCC 9029.

Table – 2 Antibacterial activity data

Compounds (100µg/ml)	Anti-bacterial activity **Gram (+) ve** Zone of Inhibition in (mm)				Anti-bacterial activity **Gram (-) ve** Zone of Inhibition in (mm)	
	Bacillus cereus ATCC11778	*Staphylococcus aureus* ATCC9144	*Micrococcus luteus* ATCC25923	*Staphylococcus epidermidis* ATCC10987	*Klebsiella pneumonia* ATCC29212	*Escherichia coli mutant* ATCC25922
4	21	22	20	21	19	20
5	24	23	22	22	21	22
6	22	21	21	22	21	22
7	20	21	21	21	20	21
8	20	20	22	21	19	20
9	20	21	21	19	19	19
10	24	24	25	23	23	23
11	22	23	24	23	24	22
12	23	23	22	21	21	22
13	20	21	21	20	20	21
Standard (10µg/ml)	27	26	27	27	26	25
Control DMF	-	-	-	-	-	-

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Table – 3 Antifungal activity data

Compounds (100µg/ml)	Antifungal activity Zone of Inhibition in (mm)	
	Candida albicans ATCC2091	*Aspergillus niger* ATCC9029
4	19	18
5	18	17
6	19	19
7	18	18
8	18	17
9	19	18
10	20	18
11	21	20
12	18	19
13	19	18
Standard (10µg/ml)	29	28
Control DMF	-	-

RESULTS AND DISCUSSION

The structures of the newly synthesized compounds were firmly established on the basis of their IR and ^{1}H NMR analysis. Compounds (4-13) showed absences of singlet at 3.1 ppm respect to the proton of thiol (SH) indicates the formation cyclic structure. In the IR spectra of the 3-mercapto-5-pyridin-3'yl-[1, 2, 4]-triazole, the characteristic $–NH_2$ and SH bands appeared in the region of 1340-1250 $cm^{-1}$and 2600-2550 cm^{-1} respectively. The strong bands at 1600-1430 cm^{-1} corresponding to initial NH_2 were absent, which was the most characteristic evidence of the cyclocondensation. In general, all the compounds showed significant anti-microbial activity when compared to that of standards. In summary a new series of potentially bioactive 1, 2, 4-triazole derivatives have been synthesized, having anti-microbial activities.

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REFERENCES

[1] Kurtzer F, Katritzky A R, Boulton A J, Advances in Heterocyclic Chemistry, (Academic Press, New York), 1965; 5: 165.
[2] Shivarama Holla B, Narayana Poojary K, Sooryanarayana Rao B & Shivananda M K. Eur J Med Chem 2002; 37: 511.
[3] Cooper K & Steele J, EP 329357. Chem Abstr 1990; 112: 76957.
[4] Todoulou O G, Papadaki- Valiraki A E, Ikeda S & De Clercq E. Eur J Med Chem 1994; 29: 611.

[5] Heindel N D & Reid J R. J Het Chem 1980; 17:1087.
[6] Walser A, Flyman T & Musan C. J Het Chem 1991; 28: 1121.
[7] Hitora T, Sajaki K, Yumamota H & Nakayama T. J Het Chem 1991; 28: 257.
[8] Kane J M, Barton B M, Dudley M W, Sorenson S M & Stueger M A. J Med Chem 1990; 33: 2772.
[9] Bardbury R H & Rivert J E. J Med Chem, 1991; 34:151.
[10] Kumamoto T, Toyooka K, Nishida M & Kubota S. Chem Pharm Bull 1990; 38:2595.
[11] Ashour P F A & Almazora S A H. Farmaco 1990; 45:1207.
[12] Heindel N D & Reid J R. J Het Chem 1980, 1087.
[13] Sivasubramanian N, Omprakash G. Int J Chem Sci 2010; 8(2): 783-790.
[14] Sivasubramanian N, Omprakash G. Int J Chem Sci 2010; 8(3): 1871-1877.
[15] Collee J G, Fraster A G, Marimion B P. Practical Medical Microbiology 13th ed, Churchill living stone, London 1989, pp.163-165.
[16] Ananthanarayanan R, Panikr J.C.K, Text book of microbiology 5th ed., Orient Longman Ltd.1987, pp. 620-621.