Synthesis and Biological Applications of Certain 1-acetamido-(benzothiazol-2'-yl)-5-aryltetrazole and Benzothiazol-2'-yl-1-ethylamine-5-aryltetrazoles

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ABSTRACT

A new series of 1-acetamido-(benzothiazol-2'-yl)-5-aryltetrazole and Benzothiazol-2'-yl-1-ethylamine-5-aryltetrazole were synthesized by [3+2] cycloaddition reaction of an organic azide with organic nitriles. The chemical structures of the synthesized compounds were confirmed by IR, ^{1}H NMR, mass spectral and elemental analysis. The compounds were screened for antibacterial, antifungal and analgesic activity. The results showed significant antifungal activity against *Aspergillus niger* and *Candida albicans* at 250 ìg/ml comparable to that of standard (ketokonazole). The analgesic activity of 1-acetamido-(benzothiazol-2'-yl)-5-benzyl-tetrazole and Benzothiazol-2'-yl-1-ethylamine-5-benzyltetrazole was found to equivalent of diclofenac by acetic acid induced writhing method at the dose of 100mg/Kg.

Key Words: Benzothiazole, Tetrazole, Analgesic, Antibacterial, Antifungal.

Introduction

Tetrazole derivatives are known for their potent antibacterial[1], antifungal[2], antipyretic[3], analgesic[4], anti-inflammatory and anticonvulsant[5] activities. Among the heterocyclic molecules apart from the tetrazoles, benzothiazoles another class of compounds were also reported to possess antibacterial[6], antifungal, analgesic[7] anti-inflammatory[8], anticonvulsant[9], antihistaminic[10], antitumor[11,12] and antimalerial[13] activities. Synthesis of both the type of molecules and their applications are well established [14,15] in the literature. It is therefore envisaged that the compounds containing both the chemical moieties may posses interesting biological activity. Interestingly benzothiazoles and tetrazoles attached through an ethylene or acetamido group are not reported in the literature. Synthesis and evaluation of molecules coupled with individual compounds having different properties has gained lot of momentum in the recent years. This has lead to the discovery of many new compounds. The objectives of the present study are to synthesize different substituted tetrazoles by direct cycloaddition between an organic azide and organic nitrile. and to identify the potential molecules by screening for their antibacterial (*Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa*), antifungal (*Aspergillus niger* and *Candida albicans*) by the disc diffusion method and analgesic activity (writhing reflex method).

2-Aminobenzothiazole was treated with chloroacetyl chloride and sodium azide to produce organic azide, this on treatment with organic nitriles to produce tetrazoleby [3+2] cycloaddition reaction. The chemical structures of the synthesized compounds were confirmed by means of IR, ^{1}H-NMR, mass spectral and elemental analysis.

Meterials and Methods

All the compounds studied in the present study were synthesized using analytical reagent grade chemicals**.** The melting points were taken in open capillary tube using Buchi melting point apparatus . The I.R Spectra of the compounds were recoded on ABB BOMEM FTIR Spectrophotometer MB serial II- Canada with KBr pellet. ^{1}H NMR spectra was recorded on 400MHz – Joel DPX using $CDCl_3$ as solvent. The chemical shifts are reported as parts per million downfield from tetra methyl silane (Me_4Si). Mass Spectra was recorded on Shimadzu GC-MS QP 5050A Japan.

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Microanalysis for CHN was performed in Heraeus CHN Rapid analyzer. TLC was used to check the purity of the compounds in pre-coated aluminum sheets (Silica gel 60 F_{254} Merck-Germany) using (7:3) chloroform: petroleum ether (40-60ºC) as mobile phase and visualized by iodine vapors. The animals used in the study for pharmacological testing were all approved by the institutional animal ethical committee (IAEC) Ref.No: IAEC-IX-15/CLBMCP/2003-2004.

Preparation of 2–Azido-N-acetamido-benzothiazole.

A mixture of chloroacetyl-2-aminobenzothiazole[6] (50mmol) and sodium azide (55mmol) in acetone (50ml) and dimethylformamide (2ml) was stirred for 24 hrs at 50-60 °C. After completion of the reaction as showed by TLC, the acetone-DMF mixture was poured in to water. The precipitate was collected and purified by recrystalisation from alcohol. Yield = 64.1%, m.p 208-210 ºC, *Rf* value = 0.57.

General Procedure for the Preparation of Tetrazole.

2-Azido-N-acetamido-benzothiazole (0.2mol) and substituted benzonitrile (0.2mol) and ammonium chloride (0.2mol) in DMF-ethanol mixture (100ml) were refluxed for 6 hrs at100 °C. After completion of the reaction as showed by TLC, the DMF-ethanol mixture was poured in to water. The precipitate was collected and purified by recrystalisation from alcohol. Characterised all the compounds by U.V, FTIR, Mass and elemental analysis. The following are the spectral characterization parameters.

1-Acetamido-(benzothiazol-2'-yl)-5-phenyl-tetrazole **2a**: Yield = 74.1%, m.p 108-110 ºC, *Rf* value = 0.75. UV $ë_{max}$ ($CHCl_3$): 275 nm. IR: 728, 765 for aromatic, 1105 for tetrazole, 1710 for C=O and 3393 for NH. ^{1}H-NMR ($CDCl_3$) δ: 8.17-8.25 (d, 2H; 4,7 Ar-H of benzothiazole, *J* = 7.8Hz), 7.94-7.86 (d, 2H; 5,6 Ar-H of benzothiazole, *J* = 7.8Hz), 6.90-7.44 (m, 5H; Ar-H), 3.98(s, 2H; CH_2), 2.46 (s, 1H; NH). GC-MS m/z: 336 (M^+), 177, 150(B), 135, 123, 77. Anal. Calcd for $C_{16}H_{12}N_6OS$: C, 57.12; H, 3.57; N, 25.0. Found: C, 57.18; H, 3.60; N, 25.04.

1-Acetamido-(benzothiazol-2'-yl)-5-(4-chlorophenyl)-tetrazole **2b**: Yield = 70.27%, m.p 136-138 ºC, *Rf* value = 0.64. UV $ë_{max}$ ($CHCl_3$): 251 nm. IR: 733,768 for aromatic, 1105 for tetrazole, 1686 for C=O and 3396 for NH. ^{1}H-NMR ($CDCl_3$) δ: 8.41-8.43 (d, 2H; 4,7 Ar-H of benzothiazole, *J* = 7.8Hz), 8.19-8.21 (d, 2H; 5,6 BT, *J* = 7.8Hz), 7.83-7.99 (m, 4H; Ar-H), 3.92 (s, 2H; CH_2), 2.49 (s, 1H; NH). GC-MS m/z: 370 (M^+), 283, 176, 150(B), 123, 77. Anal. Calcd for $C_{16}H_{11}ClN_6OS$: C, 51.82; H, 2.99; N, 22.66. Found: C, 51.86; H, 2.90; N, 22.74.

1-Acetamido-(benzothiazol-2'-yl)-5-(2-chlorophenyl)-tetrazole **2c**: Yield = 74.4%, m.p 98-100 ºC, *Rf* value = 0.57. UV $ë_{max}$ ($CHCl_3$): 265 nm. IR: 754, 768 for aromatic, 1105 for tetrazole, 1686 for C=O and 3395 for NH. ^{1}H-NMR ($CDCl_3$) δ: 7.95-7.99 (d, 2H; 4,7 BT, *J* = 7.8Hz), 7.73-7.75 (d, 2H; 5,6 BT, *J* = 7.8Hz), 7.30-7.45 (m, 4H; Ar-H), 3.88 (s, 2H; CH_2), 2.48 (s, 1H; NH). GC-MS m/z: 370(M^+), 233, 177, 150(B), 135, 123, 77. Anal. Calcd for $C_{16}H_{11}ClN_6OS$: C, 51.82; H, 2.99; N, 22.66. Found: C, 51.86; H, 2.92; N, 22.64.

1-Acetamido-(benzothiazol-2'-yl)-5-benzyl-tetrazole **2d**: Yield = 51.42%, m.p 178-180 ºC, *Rf* value = 0.71. UV $ë_{max}$ ($CHCl_3$): 284 nm. IR: 728, 764 for aromatic, 1105 for tetrazole, 1710 for C=O and 3393 for NH. ^{1}H-NMR ($CDCl_3$) δ: 7.97-7.99 (d, 2H; 4,7 Ar-H of benzothiazole, *J* = 7.8Hz), 7.73-7.75 (d, 2H; 5,6 BT, *J* = 7.8Hz), 7.46-7.30 (m, 5H; Ar-H), 3.89 (s, 2H; CH_2), 2.49 (s, 1H; NH). GC-MS m/z: 350 (M^+), 233, 177, 150(B), 123, 77. Anal. Calcd for $C_{17}H_{14}N_6OS$: C, 58.27; H, 4.03; N, 24.0. Found: C, 58.22; H, 3.96; N, 24.04.

1-Acetamido-(benzothiazol-2'-yl)-5-(4-chlorobenzyl)-tetrazole **2e**: Yield = 54.1%, m.p 158-160 ºC, *Rf* value = 0.67. UV $ë_{max}$ ($CHCl_3$): 257 nm. IR: 728, 768 for aromatic, 1105 for tetrazole, 1695 for C=O and 3398 for NH. ^{1}H-NMR ($CDCl_3$) δ: 7.97-7.99 (d, 2H; 4,7 Ar-H of benzothiazole, *J* = 7.8Hz), 7.73-7.76 (d, 2H; 5,6 BT, *J* = 7.8Hz), 7.48-7.31 (m, 4H; Ar-H), 4.02-4.29 (d, 2H; CH_2), 3.93 (s, 2H; CH_2), 2.47 (s, 1H; NH). GC-MS m/z: 384(M^+), 233, 177, 150(B), 135, 123, 77. Anal. Calcd for $C_{17}H_{13}ClN_6OS$: C, 53.05; H, 3.40; N, 21.84. Found: C, 53.20; H, 3.42; N, 21.96.

Benzothiazol-2'-yl-1-ethylamine-5-phenyltetrazole **2f**: Yield = 44.5%, m.p 78-80 ºC, *Rf* value = 0.67. UV $ë_{max}$ ($CHCl_3$): 254 nm. IR: 719, 741 for aromatic, 1105 for tetrazole and 3395 for NH. ^{1}H-NMR ($CDCl_3$) δ: 7.86-7.88 (d, 2H; 4,7 BT, *J* = 7.8Hz), 7.71-7.68 (d, 2H; 5,6 BT, *J* = 7.8Hz), 6.98-7.30 (m, 5H; Ar-H), 4.16, 3.95 (dd, 4H; $2CH_2$, *J* = 13.66Hz), 2.38 (s, 1H; NH). GC-MS m/z: 322 (M^+), 240, 150(B), 123, 77. Anal. Calcd for $C_{16}H_{14}N_6S$: C, 59.60; H, 4.38; N, 26.07. Found: C, 59.68; H, 4.30; N, 26.04.

Benzothiazol-2'-yl-1-ethylamine-5-(4-chlorophenyl)tetrazole **2g**: Yield = 49.15%, m.p 108-110 ºC, *Rf* value = 0.62. UV $ë_{max}$ ($CHCl_3$): 241 nm. IR: 719, 741 for aromatic, 1105 for tetrazole and 3398 for NH. ^{1}H-NMR ($CDCl_3$) δ: 7.86-7.88 (d, 2H; 4,7 Ar-H of benzothiazole, *J* = 7.8Hz), 7.64-7.66 (d, 2H; 5,6 Ar-H of benzothiazole, *J* = 7.8Hz), 6.97-7.44 (m, 4H; Ar-H), 4.18, 3.99 (dd, 4H; $2CH_2$, *J* = 13.66Hz), 2.48 (s, 1H; NH). GC-MS m/z: 356 (M^+), 275, 228, 150(B), 123, 77. Anal. Calcd for $C_{16}H_{13}ClN_6S$: C, 53.85; H, 3.67; N, 23.56. Found: C, 53.98; H, 3.68; N, 23.62.

Benzothiazol-2'-yl-1-ethylamine-5-(2-chlorophenyl)tetrazole **2h**: Yield = 47.75%, m.p 88-90 ºC, *Rf* value = 0.55. UV $ë_{max}$ ($CHCl_3$): 232 nm. IR: 719, 741 for aromatic, 1105 for tetrazole and 3396 for NH. ^{1}H-NMR ($CDCl_3$) δ: 7.61-7.63 (d, 2H; 4,7 Ar-H of benzothiazole, *J* = 7.8Hz), 7.30-7.32 (d, 2H; 5,6 BT, *J* = 7.8Hz), 6.96-7.20 (m, 4H; Ar-H), 4.16, 3.96 (dd, 4H; $2CH_2$, *J* = 13.66Hz), 2.47 (s, 1H; NH). GC-MS m/z: 356 (M^+), 275, 228, 150(B), 123, 77. Anal. Calcd for $C_{16}H_{13}ClN_6S$: C, 53.85; H, 3.67; N, 23.56. Found: C, 53.58; H, 3.60; N, 23.54.

Benzothiazol-2'-yl-1-ethylamine-5-benzyltetrazole **2i**: Yield = 35.29%, m.p 108-110 ºC, *Rf* value = 0.66. UV $\ddot{e}_{max}$ ($CHCl_3$): 220 nm. IR: 719, 741 for aromatic, 1105 for tetrazole and 3395 for NH. ^{1}H-NMR ($CDCl_3$) δ: 7.61-7.63 (d, 2H; 4,7 Ar-H of benzothiazole, *J* = 7.8Hz), 7.30-7.32 (d, 2H; 5,6 BT, *J* = 7.8Hz), 6.96-7.20 (m, 5H; Ar-H), 4.20, 4.06, 3.89 (ddd, 6H; $3CH_2$ *J* = 13.66Hz), 2.45 (s, 1H; NH). GC-MS m/z: 337 (M^+), 256, 150(B), 123, 77. Anal. Calcd for $C_{17}H_{16}N_6S$: C, 60.69; H, 4.79; N, 25.0. Found: C, 60.68; H, 4.70; N, 25.04.

Benzothiazol-2'-yl-1-ethylamine-5-(4-chlorobenzyl)tetrazole **2j**: Yield = 25.5%, m.p 95-97 ºC, *Rf* value = 0.62. UV $\ddot{e}_{max}$ ($CHCl_3$): 230 nm. IR: 720,742 for aromatic, 1105 for tetrazole and 3398 for NH. ^{1}H-NMR ($CDCl_3$) δ: 7.80-7.82 (d, 2H; 4,7 Ar-H of benzothiazole, *J* = 7.8Hz), 7.72-7.74 (d, 2H; 5,6 Ar-H of benzothiazole, *J* = 7.8Hz), 6.94-7.44 (m, 4H; Ar-H), 4.21, 3.99, 3.86 (ddd, 6H; $3CH_2$ *J* = 13.66Hz) 2.49 (s, 1H; NH). GC-MS m/z: 370 (M^+), 290, 150(B), 123, 77. Anal. Calcd for $C_{17}H_{15}ClN_6S$: C, 55.05; H, 4.08; N, 22.66. Found: C, 55.18; H, 3.90; N, 22.76. All the compounds gave satisfactory chemical analysis (± 0.4%).

Biological Activity of prepared compounds

1. Antibacterial activity

The antibacterial activity of synthesized compounds was studied by the disc diffusion method[17,18] against *Staphylococcus aureus* NCCS 2079 *Bacillus cereus* NCCS 2106 (gram positive) and *Escherichia coli* NCCS2065 and *Pseudomonas aeruginosa* NCCS2200 (gram negative).

The synthesized compounds were used in the concentration of 250 µg/ml using DMSO as a solvent. The Amoxycillin 10 µg/disc and Cefaclor 30µg/disc were used as a standard. (Himedia laboratories limited, Mumbai). The minimum inhibitory concentration (MIC) was determined by the test tube dilution technique using Mueller-Hinton nutrient broth method. The *in vitro* antibacterial activity showed good activity when compared with that of standard. The results are presented in table I.

2. Antifungal activity

The antifungal activity of synthesized compounds were studied by disc diffusion method[17,18] against *Aspergillus niger* NCCS 1196 and *Candida albicans* NCCS 3471. The synthesized compounds were tested in the concentrations of 250 µg/ml using DMSO as a solvent. The standard used was ketaconazole 100 µg/ml against both the organisms. The results are presented in table I.

3. Analgesic activity[19]

The analgesic activity was determined by acetic acid induced writhing method. Wister albino mice (n = 6) of either sex selected by random sampling technique were used for the study. Diclofenac sodium at the dose of 25 mg/

Table I:
Antibacterial, antifungal and Analgesic activity of the compounds

Compound	Antibacterial activity				Antifungal activity		Analgesic activity	
	Zone of inhibition (MIC)				Zone of inhibition (MIC)		Mean	% Protection
	S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*	*A. Niger*	*C. Albicans*	Writhings ± SEM	
1	19(70)	18(95)	20(65)	22(60)	23	27	11.16 ± 0.76*	76.34
2	21(50)	19(75)	22(60)	24(50)	20	24	11.34 ± 1.19*	75.95
3	17(75)	16(120)	19(75)	20(60)	18	20	12.66 ± 0.88*	73.4
4	20(70)	19(80)	21(70)	22(60)	22	27	9.5 ± 0.6*	80.05
5	21(70)	20(75)	23(50)	23(55)	23	25	10.16 ± 1.01*	78.46
6	23(60)	18(100)	22(75)	21(70)	24	29	11.83 ± 0.71*	74.92
7	24(50)	20(80)	22(70)	23(60)	22	26	15.5 ± 0.76*	67.14
8	21(60)	16(125)	20(60)	19(75)	19	21	14.33 ± 0.70*	69.62
9	22(50)	19(85)	21(65)	20(65)	24	28	9.5 ± 0.84*	80.05
10	23(50)	20(75)	23(70)	22(60)	23	27	10.85 ± 0.88*	77.0
Cefaclor	19	22	19	20	-	-	-	-
Amoxycillin	21	27	24	22	-	-	-	-
Ketoconazole	-	-	-	-	22	25	-	-
Control	-	-	-	-	-	-	47.16 ± 0.87	-
Diclofenac	-	-	-	-	-	-	7.66 ± 0.68*	83.9

Zone of Inhibition in mm & MIC in µg/ml
Significance levels: $^*p < 0.001$ compared to control

kg was administered as standard drug for comparison. All the synthesized compounds were administered to the animals (100 mg/kg) by oral route through 1%CMC. The negative control received only the solvent (1%CMC). After 30 minutes of drug administration each mice in all the groups were injected with 0.1ml of 0.6%v/v acetic acid by intraperitoneally. Calculated the number of observed writhings for each animal for a period of 20 minutes after administration of acetic acid and percentage protection was calculated for analgesic activity by compared with the control. The results are analyzed statistically by student "t" test and presented in table I.

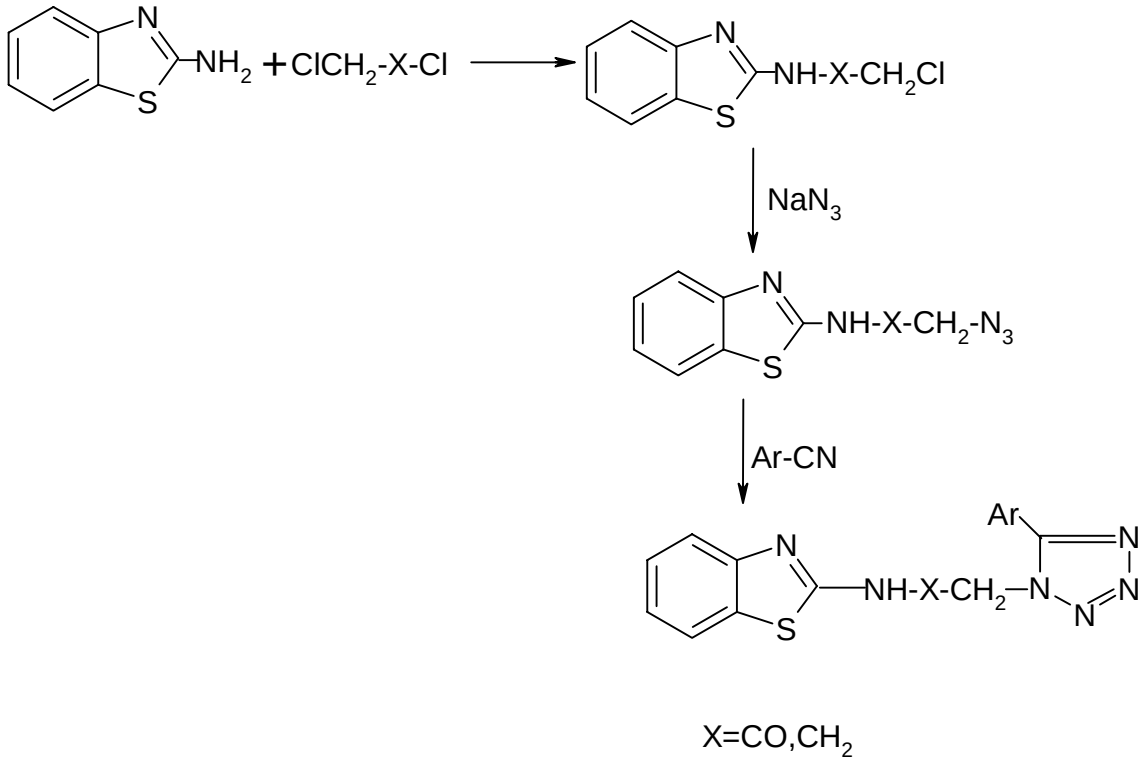
% Protection = 100 – [(experimental/control)×100]

Results and Discussion

Aminobenzothiazole on acetylation with chloroacetylchloride resulted 2 –chloroacetyl-aminobenzothiazole[6] which on further reactiion with sodium azide gave 2-azido-acetyl-aminobenzothiazole[16]. Cycloaddition of 2-azido-acetyl-amino benzo-thiazole[16] with substituted aromatic nitriles resulted 1-acetamido-(benzothiazol-2'-yl)-5-phenyl-tetrazole. The same procedure was followed for the preparation of Benzothiazol-2'-yl-1-ethylamine-5-phenyltetrazole, instead of chloroacetylchloride the 1,2 dichloroethane was used.

The structure of the synthesized compounds was characterized by IR, ^{1}H-NMR, mass spectral and elemental analysis. Infra red spectrum of the synthesized compound showed absorption bands at 1105 for tetrazole ring and 3393- 3398 for NH stretching. ^{1}H NMR spectra of the synthesized compounds produced singlet at 2.4 for NH protons, doublets between 4.0-4.2 for CH_2 for protons and multiplet between 7.1 -8.5 for aromatic protons. The expected signals with appropriate multiplicities for different types of protons were observed for the derivatives. Mass spectra of the all compounds produced clear M^+ ion peak for all the investigated molecules , and fragment ion peaks due to the cleavage of benzothiazole (m/z 150) and phenyl (m/z 77) group .

SYNTHEIC SCHEME



Ar =C_6H_5, p-ClC_6H_4, o-ClC_6H_4, $C_6H_5CH_2$, p-$ClC_6H_4CH_2$

FIG. 1: Synthetic scheme of 1-acetamido-(benzothiazol-2'-yl)-5-benzyl-tetrazole and Benzothiazol-2'-yl-1-ethylamine-5-benzyl-tetrazole

The synthesized compounds showed good antibacterial, antifungal and analgesic activity. The most prominent antibacterial activity can be seen with 1-acetamido-(benzothiazol-2'-yl)-5-(4-chlorophenyl)-tetrazole and significant antifungal activity with phenyl or benzyl group substituted tetrazole. All the tested compounds exhibited good analgesic activity at 100 mg/kg dose by oral route with 1% CMC when compared with standard (Diclofenac sodium). The analgesic activity of 1-acetamido-(benzothiazol-2'-yl)-5-benzyl-tetrazole and Benzothiazol-2'-yl-1-ethylamine-5-benzyl-tetrazole by acetic acid induced writhing method at the dose of 100mg/Kg was found to equivalent of diclofenac 25mg/Kg..

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Evaluation of anitimicrobial activities of *Cleistanthus Collinus* (RXB). Benth. & Hook. F.

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ABSTRACT

The present study was designated to evaluate the antimicrobial activities of acetone soluble , ethyl acetate , acetone insoluble , butanone , butyl alcohol fractions from the methanolic extract of the bark of *Cleistanthus collinus*, which is a small, rarely moderate sized tree considered a useful application in cutaneous diseases. The antimicrobial activities of the extracts against 6 bacterial species and 2 fungal strains were tested by using Cup plate agar diffusion method. The results showed that all the fractions had no antifungal but exhibited antibacterial activity and dose dependent. Of all the fractions, only butanone fraction exhibited broad antibacterial activity.

KEYWORDS: *Cleistanthus collinus* ; bark; antibacterial and antifungal activities.

Introduction

Cleistanthus collinus [Family: Euphorbiaceae] (local name: Nalla kodise) is a poisonous plant [1] which yields hard and durable heartwood useful for agricultural implements [2]. It is native to India, Malaysia, and Africa [3] and grown in plains, waste lands, near water streams in deciduous forests [4]. Many parts of the plant are reported to be toxic, and the extract of crushed leaves is used as a cattle and fish poison, abortifacient, and in suicide and homicide attempts [5]. For the severe headache, the head and upper part of the body are bathed in water in which the leaves have been steeped. In Chota Nagapur, the fruit and bark are employed to poison fish. The bark is beneficial in skin diseases [6, 7].

There are very few published reports on the clinical and metabolic effects of this toxic compound in human beings [8,9]. It was reported to posses antifertility [10], anticancer activity [11]. Leucoanthocyanidins [12], Arylnaphthalide Lignans [13] have been isolated. The fruits were shown to contain sitosterol and lupeol [14]. Therefore, in view of traditional use in skin diseases, we carried out a screening of bark of *Cleistanthus collinus* against pathogenic bacteria and fungi in ordered to detect new sources of antimicrobial agents.

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Materials And Methods

Plant material

The plant (*Cleistanthus collinus)* growing in Medak Dist, Andhra pradesh, India was authenticated by Prof. Raju S. Vastavaya, Taxonamist, Department of Botany, Kakatiya University, Warangal. A voucher specimen (BCC-057) was deposited at the herbarium of Ucpsc, KU, Warangal.

Extraction and isolation

The bark of the plant was peeled off and shade dried at room temperature and ground in a power mill. The powder was extracted thrice with methanol by maceration. The extract was filtered through a Buchner funnel with Whatmann number 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator. So obtained extract was defatted with petroleum ether followed by triturated with acetone and filtered to get acetone soluble and insoluble fractions. Both of them were dried by keeping at room temperature. Acetone soluble fraction suspended in water and extracted repeatedly with ethyl acetate. The ethyl acetate extract and remaining acetone soluble portion were dried. Acetone insoluble portion suspended in water and successively extracted with butanone and butyl alcohol. Then they were dried. The fractions obtained were stored at -20°C until being used. Preliminary phytochemical investigations of the extracts conducted as per the procedures described by Kokate [15] and revealed the presence of tannins, glycosides, phenols and terpenoids.

Test microorganisms

The bacterial and fungal strains used for the screening were Gram-negative bacteria such as *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus putida,* Gram-positive bacteria like *Staphylococcus aureus, Bacillus subtalis* and fungi such as *Candida albicans, Cryptococcus neoformans*. They were obtained from department of Microbiology, Kakatiya University, Warangal.

Antimicrobial assay

Antimicrobial activity was carried out using Cup plate agar diffusion method [16]. Petri plates were prepared with 25 ml of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai) for bacteria and 25 ml of Sorbitol Dextrose Agar (SDA) for fungi. The tests were dissolved in dimethyl sulphoxide and activity conducted at three different concentrations of the extract (1.25, 2.5, 5 mg per cavity) with three replicates. Negative control was prepared using respective solvent. Streptomycin (10ìg/cavity) was used as positive control. The plates were incubated for 24 h at 37° C for bacteria and 48 h at 27° C for fungi. Zone of inhibition was recorded in millimeters.

Results

The antimicrobial activities of *Cleistanthus collinus* (acetone soluble, ethyl acetate, acetone insoluble, butanone and butyl alcohol fractions from the methanolic extract) against microorganisms examined in the present study and their potency was qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameter. The results were given in Table 1. The maximal inhibition zones for bacterial strains, which were sensitive to *Cleistanthus collinus* fractions, were in the range of 10–17 mm. In the case of the butanone fraction, the maximal inhibition zones of the bacterial strains sensitive to the same were 12–17mm. In the case of the acetone soluble, ethyl acetate, acetone insoluble and butyl alcohol fraction, the maximal inhibition zones were 14-15mm, 13-16mm, 13-15mm, 10-13mm respectively.

Table 1.

Antibacterial activity of different solvent extracts of *Cleistanthus collinus* bark

Extracts	Concentration	Agar diffusion method (inhibition zone, mm)						
(mg/disc)		E.c	K.p	P.a	P.p	S.a	B.s	C.a	C.n
Acetone soluble fraction	1.25	-	-	10	8	10	-	-
	2.5	-	-	14	12	14	—	-
	5	-	-	15	14	15	—	-
Ethyl acetate fraction	1.25	-	8	10	12	-	-	-
	2.5	-	12	12	14	-	-	-
	5	-	14	13	16	-	-	-
Acetone insoluble fraction	1.25	-	8	11	10	—	-	-
	2.5	-	12	12	12	—	-	-
	5	-	13	15	13	—	-	-
Butanone fraction	1.25	10	10	8	8	12	0	–
	2.5	13	12	11	10	14	10	–
	5	17	15	14	15	17	12	–
Butyl alcohol fraction	1.25	-	-	-	-	10	-	--
	2.5	-	-	-	-	12	08	-
	5	-	-	-	-	13	10	–
Streptomycin	10ìg	25	19	21	27	20	15	–

Cup diameter 6mm

-, no activity; E.c, *Escherichia coli;* K.p, *Klebsiella pneumonia;* P.a, *Pseudomonas aeruginosa;* P.p, *Proteus putida;* S.a, *Staphylococcus aureus;* B.s, *Bacillus subtalis; C.a, Candida albican; C.n, Cryptococcus neoformans;* Streptomycin, control antibiotics.

Discussion

Qualitative analysis of different solvent extract of *Cleistanthus collinus* revealed the presence of tannins, glycosides, phenols and terpenoids which may be responsible for the observed antibacterial property of *C. collinus.* The results showed that all the fractions had no antifungal but exhibited antibacterial activity and dose dependent. Of all the fractions, only butanone fraction exhibited broad antibacterial activity. Acetone soluble fraction has antibactreial activity against two gram negative and one gram positive bacteria. Ethyl acetate and acetone insoluble fractions showed activity against gram negative and butyl alcohol fraction showed activity against gram positive bacteria.

Based on these results, it is possible to conclude that butanone extract has stronger and broader spectrum of antimicrobial activity as compared to other fractions. Findings in this study supported the traditional uses of *Cleistanthus collinus.* Therefore, this result may suggest that fractions possess compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases in human.

Acknowledgements

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"Antihyperlipidemic Effect of *Derris Trifoliata* Lour in Triton Induced Hyperlipidemia in Rats"

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ABSTRACT

The plant *Derris trifoliata Lour* is a large woody climber, which is distributed worldwide. *Derris* species is traditionally being used as a pesticide and reported for its anti Inflammatory, antioxidant, anticancer and antimicrobial activity. In the present study, the methanolic extract of leaves and stems of *Derris trifoliata* Lour were evaluated for its hypocholesterolaemic and hypoglyceridaemic effect using Triton WR-1339 induced hyperlipidemic rats as experimental model.

The group of animals treated with methanolic extract of *Derris trifoliata* Lour exhibited a significant decrease ($p<0.01$) in levels of triglycerides and cholesterol after 7 hrs as compared to the control group. The level of HDL also significantly increased ($p<0.01$) in *Derris trifoliata* Lour treated groups after 7 hrs as compared to the standard drug fenofibrate. Hence it can be concluded that *Derris trifoliata* Lour, has significant antihyperlipidemic effect owing to its ability to reduce the levels of total cholesterol, triglyceride with an increases in the level of HDL.

Key words: - Hyperlipidemia, *Derris trifoliata* Lour, Triton WR 1339.

Introduction

Increased plasma lipid levels, mainly total cholesterol (TC), triglycerides (TG), along with decrease in high density lipoproteins (HDL) are known to cause hyperlipidemia which is core in the initiation and progression of arthrosclerosis impasse. The prime consideration in therapy for hyperlipidemia is to enervate the elevated plasma levels of TC and TG along with increase in HDL lipids levels. The ideal approach to prevent or to treat arthrosclerosis and CVS complications is to target the lipid profile of hyperlipidemic patients using lipid lowering drugs or by improving the diet.[1]

Derris trifoliata Lour is a genus of *Derris* species and belongs to Leguminosae family. It is a mangrove associated plant found in the tropical and sub-tropical areas worldwide.

Chemically the roots of *Derris trifoliata* Lour contain alkaloids, carbohydrates, flavonoids and flavonols, glycosides, lipids, polysaccharides, proteins, rotenone, steroids and triterpenoids, tannins, saponins and sugars. Traditionally, it is used as a stimulant, spasmodic, counter irritant, laxative, fish poison and pesticide. It is tested for its toxicity to fish[2, 3]. It is also used to treat calculus and asthma. Rotenone, one of its active constituent is reported for its anti cancer activity[4].

However literature survey reveals no report of the antihyperlipidemic activity of *Derris trifoliata* Lour. Hence the present study was designed to investigate the effect of *Derris trifoliata* Lour on Triton WR 1339 induced hyperlipidemia in rats.

Materials and Method

Collection and extraction:

The fresh leaves and stem of *Derris trifoliata* lour was collected from mature plants growing near Vengurla sea face region; district Sindhudurga, Maharastra, India. Its botanical identification was confirmed by Botanical survey of India, Pune.

(Voucher specimen number BSI/WC/TECH/2005/1146). The air-dried leaves and stems (1.kg) of *Derris trifoliata* were powdered in a Wiley mill and soaked with methanol (5 lit.) for 18 hrs and extracted on a water bath for 6 hrs. The

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extract was concentrated under vacuum to obtain a residue of 86.24 g (8.6 % W/W). The residue obtained after concentration, gave positive Liebermann-Bruchard reaction for sterols and triterpenoids, and a positive test for flavonoids (Ferric Chloride test and Shinoda's test).

Drugs

Fenofibrate (Zydus Cadila), Triton WR 1339 (Sigma Aldrich USA), Carboxy methyl cellulose (CMC) and other chemicals used were of analytical grade. Fenofibrate was administered orally in saline solution, the methanolic extract was administered as an aqueous suspension in 1% cmc, and Triton WR 1339 was injected i.p. in saline solution.

Animals

Wistar rats of either sex weighing 150–180 g used for experiments were obtained from National Toxicological Center, Pune. Animals were housed in controlled room with 12 hr light and dark cycle at room temperature and feed with standard chow diet and water.

Experimental animal protocol [1, 5, 6]

Experimental rats, starved for 18 hr, were provided water ad libitum. The rats were divided in 6 groups containing 5 animals each. Treatment protocol for each group was given as follows,

Group I- Normal Control (NC):- 1% CMC, (1ml/kg, p.o.)
Group II- Hyperlipidemic (HG):- Triton (200mg/kg, i.p.)
Group III-Fenofibrate (FG):- Triton (200mg/kg, i.p.) + Fenofibrate (65 mg/kg, p.o.)
Group IV- (DTE 175):- Triton (200mg/kg, i.p.) + DTE (175 mg/kg, p.o.)
Group V- (DTE 350):- Triton (200mg/kg, i.p.) + DTE (350 mg/kg, p.o.)
Group VI- (DTE 700):- Triton (200mg/kg, i.p.) + DTE (700 mg/kg, p.o.)
DTE- *Derris trifoliata* Lour extract group.

Hyperlipidemia was induced by Triton WR 1339 (200mg/kg i.p.) in group II, III, IV, V, VI. Group III received Fenofibrate (65mg/kg, p.o) while groups IV, V, VI received the *Derris trifoliata* Lour methanolic extract 175, 350 and 700 mg/kg p.o. respectively immediately after injection of Triton. In the following period of the study (48 hr) animals had access only to water.

The Institutional Ethical Committee Approval no. was DYPIPSR- Protocol- P-21.

The criterion for selection of dose of extract was based on LD_{50} values. The LD_{50} value of the methanolic extract by oral route in rats was found to be 3500mg/kg body weight. Hence 175 mg/kg (low dose), 350 mg/kg (medium dose) and 700 mg/kg (high dose) were selected for the study.

Biochemical estimation[7, 8]

Blood samples were collected after 7, 24 and 48 hr of Triton injection by retrorbital puncture. Blood was immediately centrifuged (2500 rpm for 10 min.) and serum was analyzed for total cholesterol, triglyceride and HDL level using biochemical kits.

Statistical analysis

Data obtained was analysed by unpaired "t" test and ANOVA followed by Dunett test. Values were expressed as mean ± SEM and P values < 0.05 was considered significant and P values < 0.01 was considered highly significant.

Results

Induction of hyperlipidemia with Triton WR 1339

The level of serum total cholesterol, triglyceride, HDL in groups NC, HG, DTE 175, DTE 350 and DTE 700 after 7, 24, 48 hr from treatment are reported in tables 1, 2 and 3 respectively.

In HG group, significant increase ($p<0.001$) in the level of total cholesterol and triglyceride was observed at 7, 24 and 48 hr after induction with Triton as compared to NC Group.

The increase in level of serum total cholesterol and triglyceride after 7 hr was observed to be 76.53% and 29.09 % respectively. After 24 hr, the further elevation in the levels of total cholesterol and triglyceride were found to be 192.3 % and 234.03 % respectively. After 48 hrs, the level of total cholesterol and triglycerides were found to be 66.95 % and 16.16 % respectively. No significant change in HG group was observed in levels of HDL after 7, 24, and 48 hrs.

Effect of Derris Trifoliata Lour on lipid profile in hyperlipidemic rat

Effect on triglyceride levels in serum (Table 1)

The groups treated with methanolic extract of *Derris trifoliata* Lour showed significant decrease ($p<0.01$) in the level of triglycerides at all the doses after 24 hr. However a significant decrease ($p<0.01$) was observed only in groups DTE 350 and DTE 700 after 7 hrs itself and which persisted upto 48 hr of treatment.

Effect on cholesterol level in serum (Table 2)

The groups treated with methanolic extract of *Derris trifoliata* Lour showed significant decrease ($p<0.01$) in the level of total cholesterol at all the doses after 7 and 24 hr, where as a significant decrease ($p<0.05$, $p<0.01$) in level of total cholesterol was observed in groups DTE 350 and DTE 700 after 48 hr respectively compared to standard drug finofibrate.

Effect on HDL level in serum (Table 3)

The levels of HDL significantly increased ($p<0.01$) in *Derris trifoliata* Lour treated groups DTE 350 and DTE 700 after 7 and 24 hr .However a significant increase ($p<0.01$) was observed only in Group DTE 700 after 48 hr of treatment.

Effect of fenofibrate on lipid profile

Fenofibrate (65 mg/kg, p.o.) treated group showed significant decrease ($p<0.01$) in the level of total cholesterol after 7 and 24 hr and in the levels of triglyceride after 7, 24 and 48 hr. However no significant changes were observed in the level of HDL after 7, 24 and 48 hr of treatment.

The percent reduction in the levels of serum total cholesterol and triglyceride after 7, 24 and 48 hr in Fenofibrate and *Derris trifoliata* Lour treated groups are shown in table 4.

Discussion

Systemic administration of Triton WR 1339 (ionic surfactant) in fasted rats induced hyperlipidemia. The maximum plasma triglyceride and total cholesterol were reached at 20 hr followed by decline to normal values .The plant constituents like steroids, flavonoids and saponins are reported to possess lipid lowering activity[9, 10]. The plant steroids reduce the absorption of cholesterol and thus increase fecal excretion of cholesterol. Flavonoids augment

Table 1

Effect on serum level of triglycerides

Group	After 7 hr		After 24 hr		After 48 hr	
NC	64.21		59.98		63.45	
SEM	± 1.796		±1.844		±1.242	
HG	106.12	##	265.888	##	99.94	##
SEM	±3.068		±2.029		±1.478	
Fenofibrate	81.74	**	84.678	**	83.726	**
SEM	±2.262		±1.406		±1.978	
DTE 175	99.726		218.05	**	96.576	
SEM	±1.266		±3.833		±1.971	
DTE 350	93.792	**	145.548	**	88.122	**
SEM	±2.143		±4.134		±2.364	
DTE 700	86.05	**	100.05	**	85.4	
SEM	±2.104		±2.275		±3.492	

Table 2

Effect on serum level of total cholesterol

Group	After 7 hr		After 24 hr		After 48 hr	
NC	66.38		69.146		66.138	
SEM	± 2.098		±1.542		±1.721	
HG	132.434	##	226.642	##	103.764	##
SEM	±3.757		±2.718		±1.688	
Fenofibrate	74.034	**	77.724	**	100.714	
SEM	±2.184		±2.440		±2.222	
DTE 175	108.46	**	204.804	**	99.674	
SEM	±2.456		±4.248		±1.955	
DTE 350	90.756	**	150.53	**	95.668	*
SEM	±2.467		±3.516		±1.248	
DTE 700	78.818	**	90.352	**	90.68	**
SEM	±2.224		±2.765		±1.849	

DTE 175-*Derris trifoliata* lour *(*175 mg/kg, p.o.) HG-Hyperlipidemic Group
DTE 350- *Derris trifoliata* lour (350 mg/kg, p.o.), NC-Normal Control
DTE 700-*Derris trifoliata* lour (700 mg/kg, p.o.) SEM- Std. error of mean.
n = 5; Mean ± SEM. # $p< 0.01$ compared with control group.
* $p<0.05$, ** $p<0.01$ compared with test group.

Table 3

Effect on serum level of HDL

Group	After 7 hr		After 24 hr		After 48 hr	
NC	23.29		23.22		24.218	
SEM	± 1.514		±0.4544		±1.403	
HG	18.96	##	20.2444	##	21.638	##
SEM	± 1.283		±0.6332		±1.230	
Fenofibrate	22.678		23.756		22.386	
SEM	±1.028		±1.225		±1.380	
DTE 175	21.67		21.512		21.056	
SEM	±0.4584		±1.139		±0.8776	
DTE 350	30.11	**	37.028	**	23.066	
SEM	±1.486		±1.592		±1.457	
DTE 700	37.52	**	44.318	**	31.558	**
SEM	±1.815		±2.105		±1.864	

DTE 175-*Derris trifoliata* lour *(*175 mg/kg, p.o.)
DTE 350-*Derris trifoliata* lour (350 mg/kg, p.o.)
DTE 700-*Derris trifoliata* lour (700 mg/kg, p.o.)
HG-Hyperlipidemic Group
NC-Normal Control
SEM-Std. error of mean

n = 5; Mean ± SEM. # p< 0.01 compared with control group.
* p<0.05, ** p<0.01 compared with test group.

Table 4

Percent reduction in level of total Cholesterol and Triglyceride in Triton induced hyperlipidemic rats

Groups	After 7 hr		After 24 hr		After 48 hr	
	CH (%)	TG (%)	CH (%)	TG (%)	CH (%)	TG (%)
FG	76.53	29.09	192.3	234.03	60.95	16.16
DTE175	50.53	12.15	136.2	183.86	7.70	3.31
DTE350	63.89	17.73	160.2	196.72	11.56	11.76
DTE700	72.91	25.03	186.7	228.74	16.36	14.48

DTE 175: *Derris trifoliata* Lour *(*175 mg/kg, p.o.)
DTE 350: *Derris trifoliata* Lour (350 mg/kg, p.o.)
DTE 700: *Derris trifoliata* Lour (700 mg/kg, p.o.)
TC: Triglyceride
CH: Cholesterol
FG: Fenofibrate group (65 mg/kg, p.o.),

the activity of lecithin acyl transferase (LCAT), which regulates blood lipids. LCAT plays an important role in the incorporation of cholesterol into HDL (this may increase the level of HDL). Several studies have showed that increase in HDL is associated with decrease in cardiovascular diseases. Saponins also act as antihyperlipidemic agents by binding with cholesterol in intestinal lumen, so that cholesterol is less readily absorbed, besides increasing lipoprotein lipase activity which helps in removal of VLDL from the circulation.

In the present study, a decrease in serum triglyceride and cholesterol levels in groups of rats treated with methanolic extract of *Derris trifoliata. Derris trifoliata* Lour may act by inhibiting cholesterol synthesis with increased excretion of cholesterol. The extract also increased the levels of HDL.

Hence it can be concluded that *Derris Trifoliata* Lour has significant antihyperlipidemic effect owing to its ability to reduce level of total cholesterol, triglyceride with an increase in HDL levels. Further research with regard to fractionation of extract, isolation, purification and characterization of active constituents responsible for antihyperlipidemic activity and elucidation of the possible biochemical mechanism is underway.

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Studies to Evaluate the Bioactivity of Three Different Sulfenyl Compounds

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ABSTRACT

The studies on bioactivity of Benzo-2- (42 -methylphenyl)-1-thia-2, 3-diazoliumbromide (I), 42 -Methylazobenzene-2-sulfenylthiocyanate (II) & 42 -Methylazobenzene-2-sulfenylcyanide (III) have revealed that all of these three sulfenyl compounds have distinct inhibitory properties against the three strains of bacteria namely *E. coli* (gram-negative), *Staphylococcus aurius* (gram-positive) & *Salmonella typhimurium* (gram-negative). The minimum inhibitory concentration (MIC) of the compounds have been determined and vis-à-vis compared with a randomly selected popular antibiotic i.e. oxytetracycline. Though the antibacterial efficacies of these test compounds are far below compared to oxytetracycline, but among these three, compound (III) is found to have the highest and compound (II) the lowest efficacy.

Key Words: Benzo-2- (42 -methylphenyl)-1-thia-2, 3-diazoliumbromide, 42 -Methylazobenzene-2-sulfenylthiocyanate, 42 -Methylazobenzene-2-sulfenylcyanide, antibacterial activity.

Introduction

Sulfenylhalides (R"S"X), sulfenylcyanides (R"S"CN) and sulfenylthiocyanates (R"S"SCN) may be considered as the derivatives of sulfenic acids (R"S"OH). Sulfenic acids though are unstable, have been frequently mentioned in relation to biological systems. From the viewpoint of biochemical interests in sulfenic acid derivatives are the natural occurrence of sulfenic acids and their derivatives, formation of sulfenyl derivatives as intermediates in biochemical reactions, solubility of many sulfenyl compounds in water and their ability to react with sterols, thiols, amino acids, peptides etc. in aqueous medium. Sulfenic acids have been proposed as the intermediates in a number of biochemical reactions including metabolic pathways. It is a central intermediate in both the reversible and irreversible redox modulation by reactive species of an increasing number of proteins involved in signal transduction and enzymatic pathways [1]. 6-Thiopurine, an antineoplastic agent, is said to be activated by cytochrome P-450 to a sulfenic acid, which is capable of binding to microsomal proteins. Much of the chemistry of penicillins is related to the stability of 2 –oxazetidine - 4´- sulfenic acids. This sulfenic acid can be converted to cephalosporins [2]. Sulfenium carriers play a role in oxidative phosphorylation [3]. The interaction of sulfenylhalides with penicillium carboxylesterase has been studied [4].

Among the selected, newly synthesized sulfenyl compounds for bioactivity studies, namely Benzo-2- (42 -methylphenyl)-1-thia-2, 3-diazolium bromide (I), 42 -Methylazobenzene-2-sulfenylthiocyanate (II) and 42 -Methylazobenzene-2-sulfenyl cyanide (III), where sulfur atoms are behaving as electrophiles, the compound (I) is found to react readily with thiols (R-SH) and a variety of amino acids like alanine, cystine, cysteine, tryptophan, tyrosine etc. in aqueous or aqueous alcoholic environment to form water insoluble sulfenamides. Therefore our assumption was that the compound might interfere with the protein synthesis inside the microbial cell and hence prevent their growth.

Materials and Method

Synthesis of sulfenyl compounds:

Aliphatic sulfenyl halides (R-S-X) are unstable and hence difficult to prepare. Sulfenylfluorides (R-S-F) are too unstable to be synthesized because of the strong oxidative

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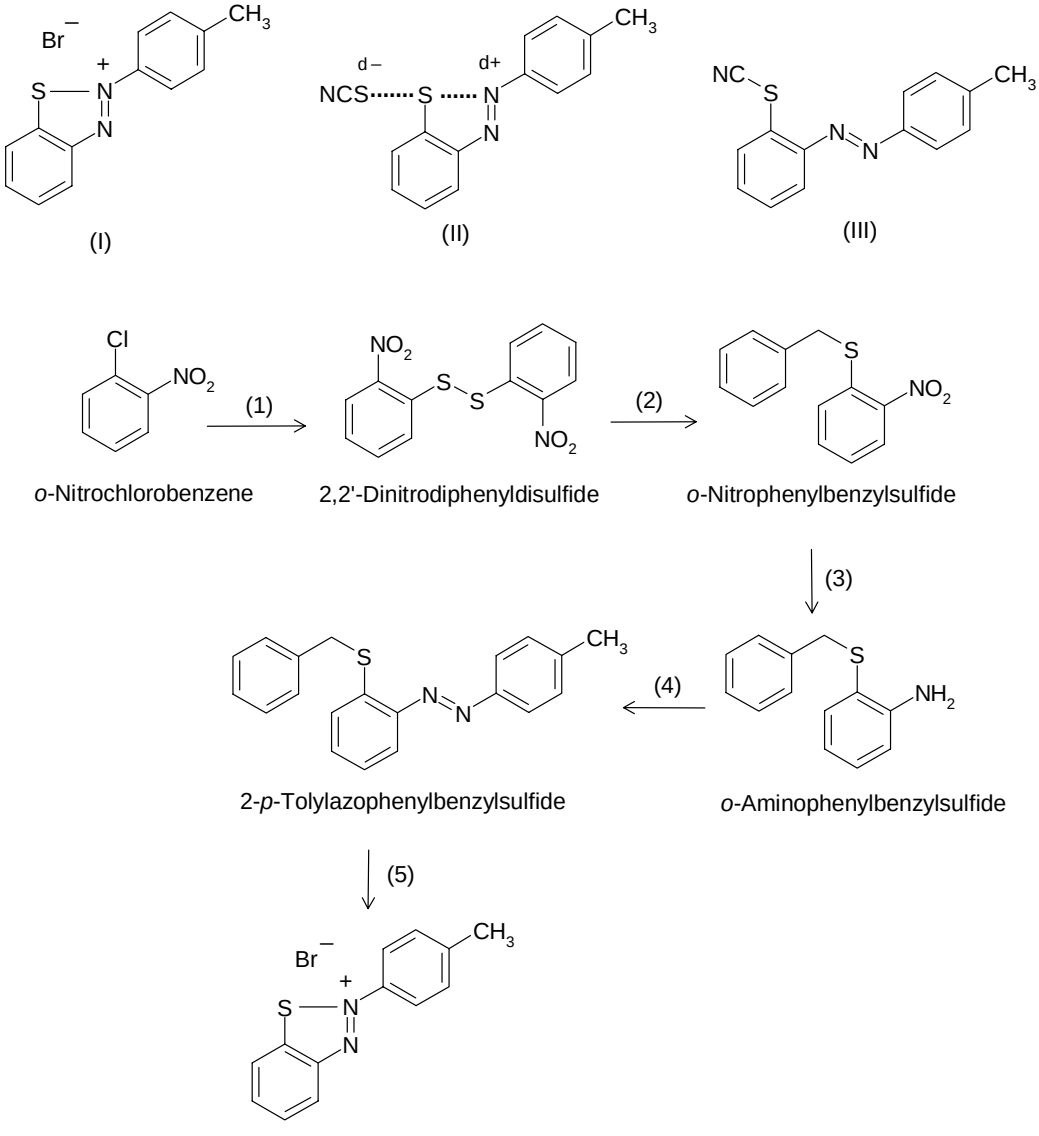
nature of fluorine and softness of divalent sulfur atom. Sulfenyliodides (R-S-I) are also too unstable due to long sulfur – iodine bond. Aliphatic Sulfenylbromides and chlorides having á- H atom are very prone to Pumerer type rearrangement [5] and hence unstable. Aromatic sulfenyl chlorides and bromides are relatively more stable due to the absence of any á- H atom and also due to d-resonance of S atom with the aromatic sextet [6]. But these are thermolabile due to dehalogenative dimerization. Computer applications to theoretically predict the thermal stability of benzenesulfenylbromides and its ortho-substituted derivatives like ortho-nitro and ortho-carboxy compounds have indicated the presence of S¯Br covalent bond in these compounds though these compounds are also thermolabile. But when an ortho-arylazo group is introduced [7], theoretical calculations show that no S¯Br covalent bond should exist in the molecule, which changes its configuration in the ground state to a stable benzo-2-aryl-1-thia-2,3-diazolium bromide (BATD-Br) salt structure. The various steps involved in the actual synthesis of compound (1) are shown in Scheme–A. Because of its salt like structure compound (I) becomes water soluble and compounds (II) and (III) are synthesized from (I) itself by the treatment of KSCN and KCN respectively in aqueous medium following simple single step nucleophilic substitution mechanism [Scheme–B]. Compound (III) exists in the pure covalent form and compound (II) assumes an intermediate form, between the pure salt like (I) and pure covalent (III) structures where both the azo and SCN groups remain linked to the sulfur atom. These facts were supported by the UV-VIS spectra and conductance studies of the compounds.



Scheme - A

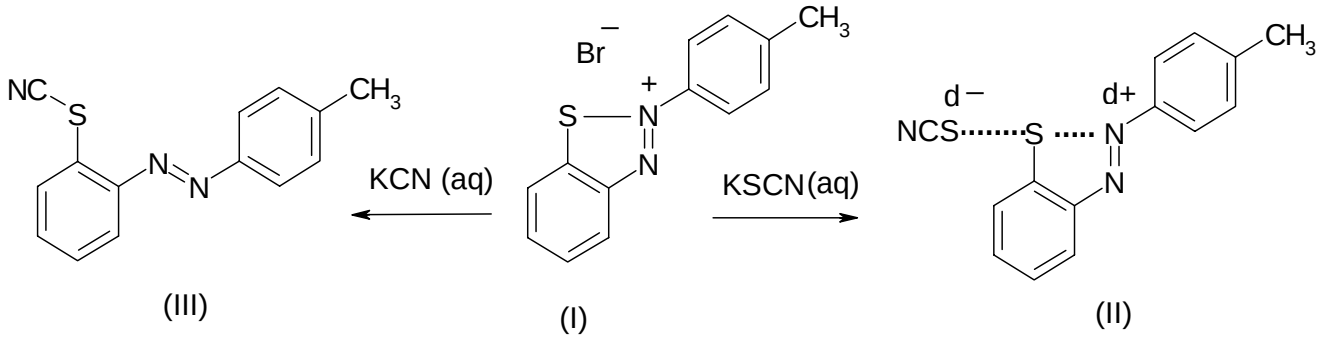
(1) Sulfur powder, Na_2S, aqueous alcohol, boil-2 hours, yellow precipitate crystallized from alcohol.

(2) Na_2S, alcohol, boil – filter, $PhCH_2Cl$ – reflux 15 min. solvent removed, yellow precipitate crystallized from alcohol.

(3) Fe, glacial CH_3COOH, $PhCH_3$ – reflux 6 hours – toluene layer collected – solvent removed – residue crystallized from light petrol 40º- 60º C as white flacks.

(4) Ar-NO, glacial CH_3COOH, heat 60º-70º C, kept overnight, orange precipitate crystallized from glacial CH_3COOH (Ar = 4-Methylphenyl-).

(5) Br_2 (equimolar), I_2 (2-3 crystals) in glacial CH_3COOH, reflux 15 min. brown precipitate crystallized from glacial CH_3COOH.



(III) (I) (II)

Scheme - B

Characterization of sulfenyl compounds:

The synthesized sulfenyl compounds were characterized by determination of melting points, UV-VIS & IR spectra and elemental C, H, N analysis. Melting points are determined using an electrical device and are uncorrected [Table –1]. The UV-VIS spectra were recorded in a Hitachi spectrophotometer [Table – 2]. The IR spectra were recorded in a Perkin – Elmer spectrophotometer [Table – 2]. The elemental analysis was performed with a Perkin – Elmer model 240 analyser [Table – 1].

Selected microorganisms:

Three pathogenic strains of bacteria i.e. *Escherichia coli, Staphylococcus aurius & Salmonella typhimurium* were selected for bioactivity studies. *Escherichia coli*, a gram–negative bacteria causes diarrhoea, *Staphylococci*, gram-positive bacteria and *Salmonella*, gram–negative bacteria causes food poisoning by producing various toxins. The strains were obtained from the Department of Microbiology, College of Veterinary Science, Khanapara, Guwahati - 22. The experiments were conducted in the department of animal production and management, College of Veterinary Science, Khanapara, Guwahati - 22. The collected strains of bacteria were maintained in Agar slants before use. For the determination of Minimum Inhibitory Concentration (MIC) the following procedures were adopted.

Preparation of test samples:

A 5% solution of sample- (I) & sample- (II) were prepared in sterile distilled water & 50% ethanol. For sample-(III) the diluted test sample could not be prepared because of the formation of precipitate between test compound and dilution fluid. Due to this precipitate the turbidity of the solution due to bacterial growth could not be read. A separate test procedure, plate test [8], was followed to determine the MIC of sample- (III).

Two-fold dilution of 5 ml solution of sample- (I) & (II) were prepared up to 10-dilutions in sterile test tube. 0.1% peptone water was used as growth promoting dilution fluid. 0.2 ml of overnight culture of above-mentioned bacterial strain was inoculated in each dilution tube in different sets. In each set of controlled tube only 0.1% sterile peptone water was taken. The tubes were incubated at 37°C for 48 hours. The concentrations of two different test compounds, which prevented the growth, as well as the turbidity of the solution were noted as MIC of that particular compound. For confirmation with naked eye reading and for demonstrating the cidal & static effect of the samples a sterile loop was used to take the sample from each dilution and inoculated into the Agar plates and incubated for 24 hours. Subculture of dilutions where very few colonies could survive was considered to contain the MIC of the samples. The different known concentrations of sample-

Table - 1

Compounds	M.P (ºC)	Yield (%)	C,H,N analysis (%)	
			Theoretical value	Value found
(I)	231 - 233	80	C= 50.8, H= 3.6, N= 9.1	C= 50.5, H= 3.1, N= 9.0
(II)	140 - 142	85	C= 58.5, H= 3.9, N= 14.7	C= 58.4, H= 4.0, N= 14.8
(III)	97 - 98	87	C= 66.4, H= 4.3, N= 16.6	C= 66.5, H= 4.5, N= 16.5

Table - 2

Compound	UV-VIS spectra					IR spectra (ν_{KBr} $^{cm^{-1}}$)
	Solvent	n? π* azo band (nm)	ε_{max}	π? π* conjugated chromophore band (nm)	ε_{max}	
(I)	Water	---	---	370	12621	3048.3 w (Ar = C-H *str*), 2999.2 and 2920.7 w (- CH_3 *str*).
	Ethanol	---	---	365	11700	
(II)	Water	---	---	342	11573	3051 w (Ar = C-H *str*), 3000 and 2928.5 w (- CH_3 *str*), 2165 m (SCN *str*).
	Ethanol	448	85	335	10500	
(III)	Water	---	---	340	11000	3052.2 w (Ar = C-H *str*), 3002.5 and 2932 w (- CH_3 *str*), 2350 s, (C=N *str*).
	Ethanol	450	110	340	11000	

(III) were incorporated in plates of Agar & streaks of different bacterial suspensions were inoculated on the surface of each plate. Agar plates are incubated for 48 hours at 37°C. The lowest concentration of sample- (III) that fully prevented the growth of bacteria was the MIC of the compound for that bacterial strain.

The strength of antibacterial activity of three samples and their respective comparison with a known antibiotic i.e. oxytetracycline equivalent were done in the following way.

Disk diffusion method of *Cruickshank* et al was applied using 30 micrograms of oxytetracycline antibiotic. Small disk of standard filter paper was impregnated with known concentration of test sample. The disks were placed on the plates of culture medium previously spread uniformly with inoculums of bacterial strains i.e. (*Escherichia coli*, *Staphylococcus aurius* & *Salmonella typhimurium*. The plates showed the zone of inhibition around the disk indicated the presence of antibacterial property of test samples. The diameter of zone of inhibition around the disk were measured and recorded in mm. The differences in inhibitory zones produced by the samples were compared with the zone of inhibition produced by the oxytetracycline. The required concentration of the samples to produce 1 mm of zone of inhibition by different test samples as well as the oxytetracycline were calculated to make a comparison and also to find out the superiority of antibacterial properties of three samples.

Results and Discussion

MIC of different compounds (sample-I & II) is presented below.

TABLE-1:

RESULTS SHOWING GROWTH OF BACTERIAL STRAINS (TUBE TEST) ON SUBCULTURING FOR DETERMINATION OF MIC OF 5% SOLUTIONS OF SAMPLE NO –I

Sample no-I (Benzo-2- (42 -methylphenyl)-1-thia-2, 3-diazoliumbromide)

Tube No	1	2	3	4	5	6	7	8	9	10
Dilution	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Concentration (mcg/ml)	25000	16600	10000	5500	2900	1510	760	380	190	90
Escherichia coli	–	–	–	–	–	–	+_	+	+	+
Staphylococcus aureus	–	–	–	–	–	+_	+	+	+	+
Salmonella typhimurium	–	–	–	–	–	+_	+	+	+	+

'+': Heavy growth
'+_': Very few colonies
'_': No growth

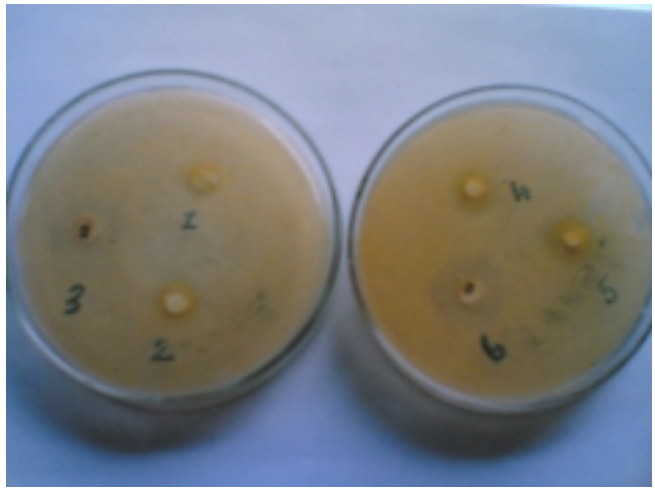


Figure showing zone of inhibition produced by different dilutions of 42 -Methylazobenzene-2-sulfenyl bromide against the bacterial strains of ***Staphylococcus aureus*** (right plate) and ***Salmonella typhimurium*** (left plate) on sub culturing performed for determination of minimum inhibitory concentration (MIC).

Left plate- (1) 250-mcg conc. of the compound.
(2) 500-mcg conc. of the compound.
(3) 30-mcg oxytetracycline.

Right plate- (4) 750-mcg conc. of the compound.
(5) 1000-mcg conc. of the compound.
(6) 30-mcg oxytetracycline.

TABLE-2:

RESULTS SHOWING GROWTH OF BACTERIAL STRAINS (TUBE TEST) ON SUBCULTURING FOR DETERMINATION OF MIC OF 5% SOLUTIONS OF SAMPLE

Sample no.-II (42 -Methylazobenzene-2-sulfenylthiocyanate)

Tube No	1	2	3	4	5	6	7	8	9	10
Dilution	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Concentration (mcg/ml)	25000	16600	10000	5500	2900	1510	760	380	190	90
Escherichia coli	–	–	–	–	–	–	+	+	+	+
Staphylococcus aureus	–	–	–	–	–	+_	+	+	+	+
Salmonella typhimurium	–	–	–	–	–	–	+	+	+	+

'+': Heavy growth
'+_': Very few colonies
'_': No growth

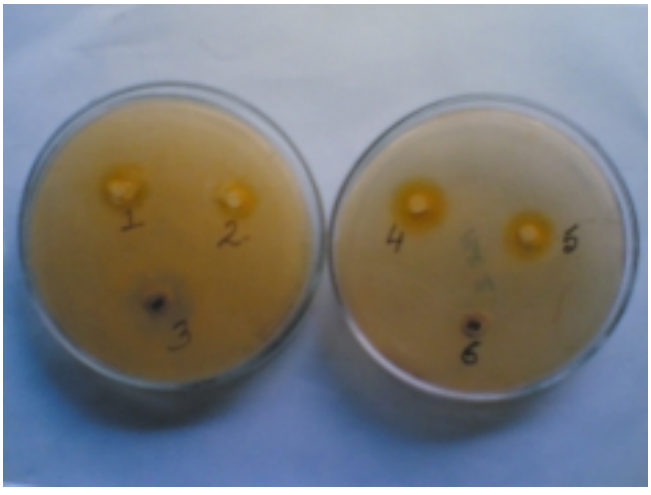


Figure showing zone of inhibition produced by different dilutions of 42 - Methylazobenzene-2-sulfenylthiocyanate against the bacterial strains of ***Staphylococcus aureus*** (left plate) and ***Escherichia coli*** (right plate) on sub culturing performed for determination of minimum inhibitory concentration (MIC).

Left plate- (1) 4% solution of the compound.
(2) 3% solution of the compound.
(3) 60-mcg oxytetracycline.

Right plate- (4) 4% solution of the compound.
(5) 3% solution of the compound.
(6) 60-mcg oxytetracycline.

Figure showing the test tubes containing 2 fold dilutions of 42 -Methylazobenzene-2-sulfenylthiocyanate and development of turbidity due to growth of ***Staphylococcus aureus*** at 1:32 dilution and onwards.

The MIC for the sample-III is presented in table-3

TABLE-3:

RESULTS SHOWING GROWTH OF BACTERIAL STRAINS (PLATE TEST) ON NUTRIENT AGAR FOR DETERMINATION OF MIC OF SAMPLE-III

Sample-III (42 -Methylazobenzene-2-sulfenylcyanide)

Plate No.	1	2	3	4	5	6	7	8	9	10
Concentration (mcg/ml)	50000	25000	20000	15000	10000	5000	2500	1000	750	500
Escherichia coli	–	–	–	–	–	–	–	+_	+	+
Staphylococcus aureus	–	–	–	–	–	–	+_	+	+	+
Salmonella typhimurium	–	–	–	–	–	–	–	+_	+	+

'+': Heavy growth
'+_': Very few colonies
'_': No growth

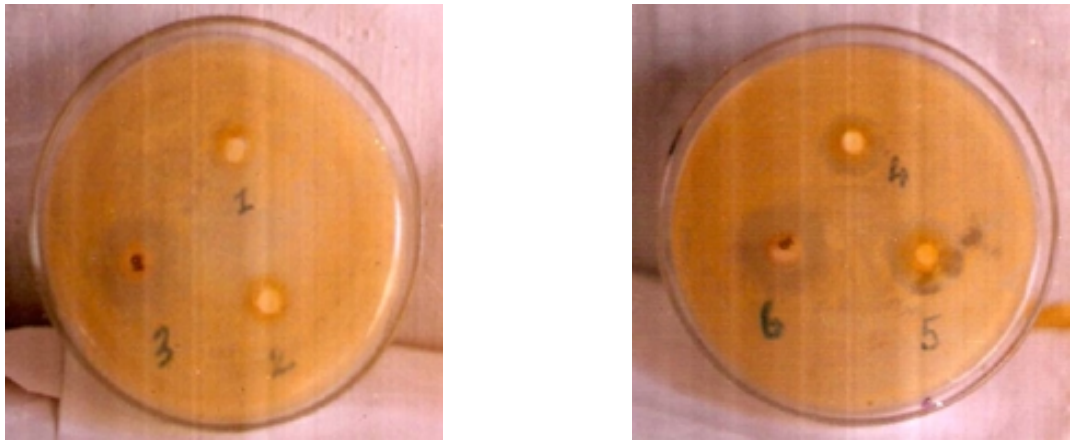


Figure showing zone of inhibition produced by different dilutions of 42 - Methylazobenzene-2-sulfenylcyanide against the bacterial strains of ***Staphylococcus aureus*** (right plate) and ***Escherichia coli*** (left plate) on sub culturing performed for determination of minimum inhibitory concentration (MIC).

Left plate-	(1)	2500 mcg/ml conc. of the compound.
	(2)	5000 mcg/ml conc. of the compound.
	(3)	30-mcg oxytetracycline.
Right plate-	(4)	5000-mcg/ml conc. of the compound.
	(5)	10000 mcg/ml conc. of the compound.
	(6)	30-mcg oxytetracycline.

Table-4:

The Strength Of Antibacterial Activity Of Different Compounds

	Sample-I		Sample-II		Sample-III		Oxytetracycline	
Test organisms	Conc. (mcg)	Zone of inhibition (mm)	Conc. (mcg)	Zone of inhibition (mm)	Conc. (mcg)	Zone of inhibition (mm)	Conc. (mcg)	Zone of inhibition (mm)
Escherichia coli	875	3.5	1750	5.0	1000	9.0	30	3.5
Salmonella typhimurium	-do-	4.0	-do-	4.5	-do-	7.0	-do-	3.5
Staphylococcus aureusa	-do-	7.0	-do-	5.0	-do-	4.0	-do-	10.0
Average	875	4.83	1750	5.0	1000	6.66	30	5.66
	1 mm zone of insibition(ZOI)= 181.15 mcg		1 mm zone of inhibition(ZOI)= 350 mcg		1 mm zone of inhibition= 150.15 mcg		1 mm zone of inhibition(ZOI)= 5.3 mcg	

So, 1 mm ZOI = 5.3 mcg of oxytetracycline ≈ 150.15 mcg of sample-III ≈ 181.15 mcg of sample-I ≈ 350 mcg of sample-II.

Or, 1 mcg of oxytetracycline ≈ 34.179 mcg of sample-I ≈ 66.03 mcg of sample-II ≈ 28.33 mcg of sample-III.

∴ Sample-III (sulfenyl cyanide) > Sample-I (sulfenyl bromide)> Sample-II (sulfenyl thiocyanate) as per antibacterial activity is concerned**.**

In addition to that it was also observed from the tables- 1,2,3 which shows the MIC of three different test samples at different concentrations that the action of sulfenyl compounds shows both static and cidal action more on gram –ve bacteria i.e. *Salmonella typhimurium* and *Escherichia coli* than on gram +ve bacteria i.e. *Staphylococcus aureus.*

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