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Synthesis and study of 2-Benzylthiobenzothiazoles and 2-Benzylthiobenzimidazoles as antituberculosis agents

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6 Methoxy-2-substituted benzyl (thio) benzothiazoles and 5, 6-dichloro-2-substituted benzylthiobenzimidazoles were synthesised by condensing the sodium salts of 6-methoxy-2-mercaptobenzthiazoles and 5, 6 dichloro 2-mercaptobenzimidazoles with substituted halides at reflux temperature using ethanol or methanol (sodium dry) as solvents. All the compounds were tested against *M. tuberculosis* H₃₇Rv strain *in vitro*.

Introduction :

As reported earlier¹⁻² compounds having a 6-methoxy-2-substituted-aryl amino benzothiazole structure have shown promising activity against *M. tuberculosis* upto 0.05 μ g. Also 5 (6) -chloro-2-substituted benzylthio-benzimidazoles² have shown activity upto 1.56 μ g/ml. In view of the above reports both the series of compounds were further investigated for their biological activity.

Chemistry :

A synthetic route for the preparation of 2-amino-6-methoxy benzothiazoles consists in thiocyanation of *p*-anisidine to get 5-methoxy-2-aminothiophenol⁴ which were further reacted with carbon disulphide to obtain 2-mercapto-6-methoxybenzothiazoles.⁵

4, 5-Dichloro-2-nitroaniline was reduced to 4, 5-dichloro-o-phenylene diamine and treated with carbon disulphide using

ethanol as solvent to get the 2-mercapto-5, 6-dichlorobenzimidazole.⁶

Sodium salt of 6-methoxy-2-mercaptobenzothiazole and 5, 6-dichloro-2-mercaptobenzimidazole were condensed with substituted benzyl and heterocyclic halides. These were prepared by the methods reported in the literature.⁷

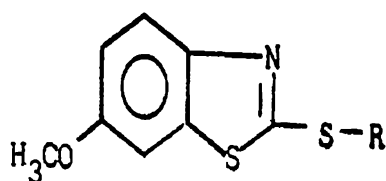
Experimental :

6-Methoxy-(3, 4-dichlorobenzylthio) benzothiazole (No. 4) :— 2-Mercapto-6-methoxybenzothiazole (1.97 g ; 0.01 mole) was dissolved in 80 ml of absolute methanol. To that sodium metal (0.11 g ; 0.01 mole) was added under anhydrous conditions. After the complete disappearance of sodium 3, 4-dichlorobenzyl bromide (2.39 g ; 0.01 mole) was added and the mixture shaken at room temperature and kept for one hour. A colourless product separated which was collected by filtration and recrystallized from ethanol, m.p. 85°C, Yield 2.5 g (75.3%).

* To whom communication to be made.

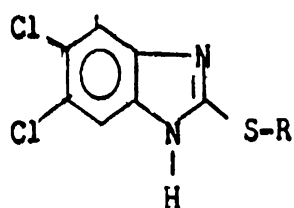
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Table 1
6-Methoxy-2-substituted-benzylthiobenzothiazoles where, R = Substituted-benzyl and heteroaryl



No.	R	M.p.°C ^a (uncorr.)	Mol. formula ^b	Yield %	Activity (μg/ml) ^c
1.	<i>p</i> -Nitrobenzyl	123	C ₁₅ H ₁₂ N ₂ O ₃ S ₂	67.85	50
2.	<i>p</i> -Chlorobenzyl	108	C ₁₅ H ₁₂ ClNOS ₂	82.20	Inactive
3.	2, 4-Dichlorobenzyl	68	C ₁₅ H ₁₁ Cl ₂ NOS ₂	40.11	100
4.	3, 4-Dichlorobenzyl	85	C ₁₅ H ₁₁ Cl ₂ NOS ₂	41.11	Inactive
5.	2-Hydroxy-5-nitrobenzyl	190	C ₁₅ H ₁₂ N ₂ O ₄ S ₂	52.00	100
6.	<i>p</i> -Bromobenzyl	120	C ₁₅ H ₁₂ BrNOS ₂	65.00	Inactive
7.	2-Bromo-4-nitrobenzyl	235	C ₁₅ H ₁₁ BrN ₂ O ₃ S ₂	60.00	12.5
8.	2-Chloro-4-nitrobenzyl	110	C ₁₅ H ₁₁ ClN ₂ O ₃ S ₂	48.00	50
9.	Benzyl	96	C ₁₅ H ₁₃ NOS ₂	76.00	12.5
10.	7-Chloroquinoline	126	C ₁₇ H ₁₁ ClN ₂ OS ₂	44.00	Inactive
11.	3, 5-Dichloropyrimidyl	120	C ₁₁ H ₇ Cl ₂ N ₂ OS ₂	83.00	12.5
12.	Pyrimidyl	142	C ₁₂ H ₉ N ₃ OS ₂	79.00	25
13.	Adamantane	166	C ₁₈ H ₂₁ NOS ₂	48.00	12.5
14.	3, 5-Dimethoxypyrimidyl	138	C ₁₄ H ₁₃ N ₃ O ₃ S ₂	38.00	—

Table 2
5, 6-Dichloro-2-substituted (thio) benzimidazoles
where, R = Substituted-benzyl and heteroaryl

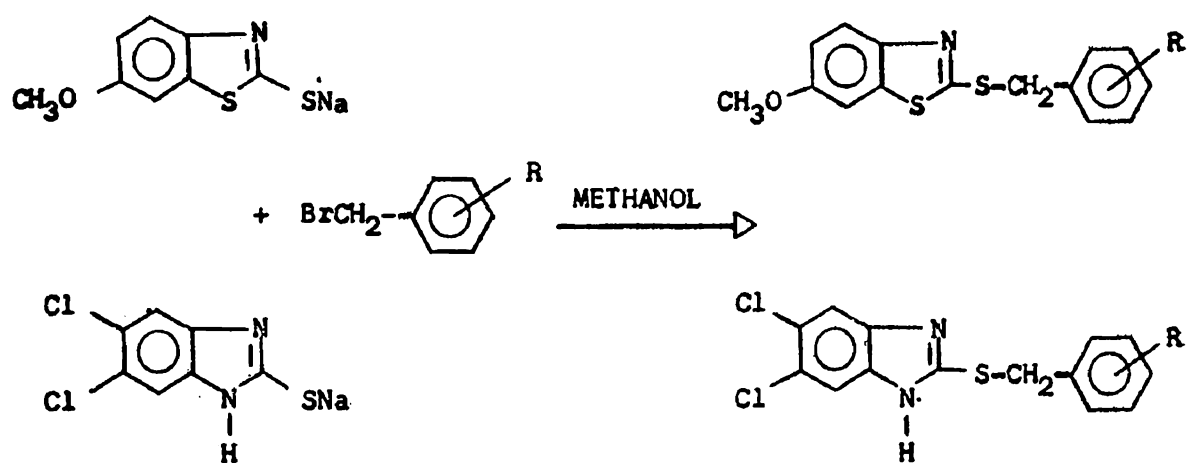


No.	R	M.p.°C ^a (uncorr.)	Mo. formula	Yield %	Activity (μg/ml) ^c
1.	Benzyl	244	C ₁₄ H ₁₀ Cl ₂ N ₂ S	66.23	—
2.	<i>p</i> -Chlorobenzyl	205	C ₁₄ H ₉ Cl ₃ N ₂ S	57.00	Inactive
3.	2, 4-Dichlorobenzyl	206	C ₁₄ H ₈ Cl ₄ N ₂ S	51.91	12.5
4.	3, 4-Dichlorobenzyl	230	C ₁₄ H ₈ Cl ₄ N ₂ S	59.57	3.12
5.	<i>p</i> -Bromobenzyl	229	C ₁₄ H ₉ BrCl ₂ N ₂ S	62.04	—
6.	2-Bromo-4-nitrobenzyl	90	C ₁₄ H ₈ BrCl ₂ N ₃ O ₂ S	50.92	—
7.	<i>p</i> -Nitrobenzyl	183	C ₁₄ H ₉ Cl ₂ N ₃ O ₂ S	54.54	—
8.	2-Hydroxy-4-nitrobenzyl	190	C ₁₄ H ₉ Cl ₂ N ₃ O ₂ S	48.91	25
9.	7-Chloroquinoline	170	C ₁₆ H ₈ Cl ₃ N ₃ S	49.00	—
10.	2-Pyrimidyl	305	C ₁₁ H ₆ Cl ₂ N ₄ S	48.64	25
11.	3, 5-Dichloropyrimidyl	93	C ₁₁ H ₄ Cl ₄ N ₄ S	41.75	—
12.	3, 5-Dimethoxy pyrimidyl	170 d.	C ₁₃ H ₁₀ Cl ₂ N ₄ O ₂ S	51.00	—
13.	Adamantane	295	C ₁₈ H ₁₆ Cl ₂ N ₂ S	43.95	—

a = Melting points taken in open capillary tubes and are uncorrected.

b = All compounds were analysed for C, H and N and the values were within $\pm 0.4\%$.

c = Minimum inhibitory concentration of standard drugs *in vitro* antituberculosis activity for INH = 0.04 mcg/ml and for PAS = 0.4 mcg/ml.

S C H E M E

Analysis for $C_{15}H_{11}Cl_2NOS_2$:

	%C	%H	%N
Calcd.	50.70	3.09	3.94
Found	50.21	3.14	4.06

5, 6-Dichloro-(2, 4-dichlorobenzylthio) benzimidazole :— A solution of the sodium salt of 5, 6-dichloro-2-mercapto-benzimidazole (2.4 g ; 0.01 mole) and 2, 4-dichlorobenzyl bromide (2.39 g ; 0.01 mole) in 30 ml of ethanol was refluxed on water-bath for 3 hours. The solvent was removed under reduced pressure and some water added. The 5, 6-dichloro-(2, 4-dichlorobenzylthio) benzimidazole precipitated out was filtered and crystallized from methanol, m.p. 206°C. Yield, 2.25 g (74.74%).

Analysis for $C_{14}H_8Cl_4N_2S$:

	%C	%H	%N
Calcd.	44.68	2.12	7.44
Found	45.12	2.48	7.29

Biological Results :

All the compounds synthesised were

tested for their *in vitro* antituberculosis activity against human virulent strain of *M. tuberculosis* (H₃₇Rv strain) by serial dilution method of Doub and Youmans.³

The antituberculosis activity shown by 2-substituted-benzylthiobenzothiazole is not much promising as compared with that of 6-methoxy-2-substituted-arylaminobenzothiazoles. The minimum inhibitory concentration shown in this series by 6-methoxy-2-benzylthiobenzothiazole, 6-methoxy-2-(2-bromo-4-nitrobenzyl) thiobenzothiazole, 6-methoxy-2-admantyl thiobenzothiazole, 6-methoxy-3, 5-dichloropyrimidyl thiobenzothiazole was found to be 12.5 µg/ml. Other compounds did not show any significant activity.

From the benzimidazole series, only 2, 4-dichloro-2-benzylthio-5, 6-dichlorobenzimidazole showed the activity upto 12.5 µg/ml, while 3, 4-dichloro-2-benzylthio derivatives exhibited activity at the dilution of 3.12 µg/ml. Other compounds were found to be inactive.

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Studies on snails in village Gimvi, a focus of human schistosomiasis in India

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A study of snail population in the area of focus of schistosomiasis situated in Gimvi village was carried out for a period of one year. Prevalence of *furcocercous cercariae* from the various species of snails was recorded.

Following snails were present in the area of rivulet:— *Ferrissia tenuis* (Bourguingant), *Paludomus obesa* (Philippi), *Indoplanorbis exustus* (Desbates) and *Melanoides tuberculatus* (Muller). The occurrence of *M. tuberculatus* was observed for first time in this area. *F. tenuis* was observed to be the commonest type of snail followed by *P. obesa*. The presence of *F. tenuis* was highest during summer months (March, April and May); while *P. obesa* was found to be prevalent throughout the year.

Introduction :

The studies described in this paper were made in the village Gimvi, situated in Guhagar Taluka of Ratnagiri district of Maharashtra State, having an estimated population of nine hundred and twelve.

The village is located in the hilly ranges of the Western Ghat of Sahyadri 250 km south of Bombay and is situated at a distance of 16 kilometers from the sea-shore.

In 1951, Gadgil and Shah¹ discovered an endemic focus of schistosomiasis in this village. In their study, they observed that eight snails of the species *Ferrissia tenuis*, the commonest snail in the area, were infected with schistosome cercariae. The infection rate in snails was 2.6%. *F. tenuis* was therefore incriminated as the intermediate host by Gadgil (1963)² and Dave and Dhage (1958)³.

Although Gadgil and Shah (1955)¹, (1956)⁵; Gaitonde *et al.* (1981)⁶ have carried out some studies of snail population in the area and incriminated *F. tenuis* as the vector, a doubt always has been raised as to the possibility of presence of another vector. Therefore, a detailed study was carried out to throw light on distribution pattern, seasonal abundance and infection rate in various snail species.

Material and Methods :

Collection of snails : The snails survey was done from September 1973 to August 1979. The population densities were studied by visiting each habitation in the middle of the month. The entire tract through which the rivulet runs was examined and demarked into three main zones i.e., A, B and C. Each zone covered approximately of 1 kilometer length.

Snails were collected by using leaf

raps and flat rigid framed net attached to a long handle as suggested by Olivier and Schneiderman (1956).⁷ The density of snail population was studied using wooden frame of one square foot as quantitative method. The wooden frame was dipped in the water and whole material was collected including mud, stones, leaves within the square and counted. The total number of snails of various species was recorded. The three main zones A, B, C were studied and mean of these indicated the snail density of particular species in that particular zone at that particular period.

Observations and Results :

Ecological features of Gimvi area :

Rivulet :— Two rivulets run through the village and these are utilised by the inhabitants of the village as source of water for drinking, bathing and washing. The village also has wells and piped water supply. Despite this, population residing in the vicinity of one of these rivulets, particularly from Jadhav Wadi, mainly depends upon the rivulet for water supply. The rivulet covers a distance of 3 kilometers from its origin and joins with another small rivulet from the other side.

It originates from the hills near the village and is surrounded by thick forest on both sides. The bottom of the rivulet is rocky at the up stream, while it becomes sandy and muddy at the down stream. The water is clean at the up stream and turns turbid down stream, particularly during premonsoon i.e., in March, April and May. The

rivulet assumes the size of a big river during the monsoon i.e., from June to September or early part of October. From the month of November onwards the stream settles down and water is hardly knee-deep and the population of snails increases from this month. The snails are seen submerged in the water attached to stones and leaves in the rivulet.

Vegetation : Apart from the thick forest on both sides of the rivulet, aquatic plants like green and brown algae are seen growing on the decaying leaves. The principal crops include rice, nachani and vegetables, etc.

Temperature : The temperature of water ranged between 29°C and 30°C in the months of March, April and May. It was 20°C in June and July. The temperature was recorded at the level of 6 inches below the surface.

The atmospheric temperature of the place varied from 20°C to 25°C during different seasons of the year.

Rainfall : The average rainfall of the village Gimvi was from 250 to 300 cm per year.

The rivulet was infested with various types of snails i.e., *Ferrissia tenuis*, *Paludomus obesa*, *Indoplanorbis exustus*, *Melanoides tuberculatus* and *Pisidium* species, while in the surrounding rice field *Pila virens* (Lamarck) was present during the monsoon. Table-I, reveals presence of different types of snails during different months. Table-II, Table-III and Table-IV present details of snail density of

various species during different months.

Ferrissia tenuis (Bourguignant): The species occurs in the shallow and turbid water of the rivulet. *F. tenuis* is seen attached to stones and decaying vegetation, especially mango leaves 2 to 100 snails per leaf. The population of *F. tenuis* increased from the month of October to May and the density ranged from 15 to 2750 per square foot (Table-II). The snail populations were very low during months of June, July, August and September (Table-I). Highest density was observed in the month of May. The eggs *F. tenuis* were seen attached to decaying vegetation in the month of May only. 1200 snails of *F. tenuis* were dissected, six were found to harbour apharyngeate *furcocercous cercariae* with eye spot and twelve were found apharyngeate *furcocercous cercariae* without an eye spot. The snail was found to be prevalent in all the areas surveyed.

Paludomus obesa (Philippi): *P. obesa* were seen attached to the big stones, decaying vegetation, especially mango leaves. Some of the snails were found buried in the mud. These snails were widely distributed all over the rivulet area and were observed throughout the year but its density was low in the months of June, July and August. This may be due to heavy rain-fall. The snail population increased gradually from September to March. The density ranged between 13 to 105 snails per square foot (Table-III). Highest density was observed in the month of March.

Six hundred snails of *P. obesa* were dissected, seven were found to harbour

apharyngeate *furcocercous cercariae* without any eye spot.

Melanoides tuberculatus (Muller): *M. tuberculatus* was sparsely distributed and observed near the extreme downstream region of the rivulet. The snails were observed on the surface area of the mud in stagnant water in March, April, and May. The density was 2 to 3 snails per square foot. No *furcocercous cercariae* were found in *M. tuberculatus*. The highest density was observed in the month of April.

Indoplanorbis exustus (Deshayes): *I. exustus* was rarely found. This species was collected from two localised areas having an approximate area of 100 sq. ft. of the rivulet in the month of May only. The snails were found to remain buried in the deep and turbid water. The eggs of *I. exustus* were attached to decomposed mango leaves and other vegetation during the months of April and May. The density of these snails in the area where they were detected was 2 to 3 snails per square foot (Table-IV). No *furcocercous cercariae* was recovered from this snail.

Pisidium species: The *Pisidium* species of bivalvia was collected from the two localised areas of the rivulet having an approximate area of 150 sq. foot. The snails were observed in sandy and muddy area of the stagnant water. *Pisidium* species was rarely found in the month of May. The density in this area was 1 to 2 bivalvia per square foot (Table-IV). It is very difficult to detect this bivalvia in the sandy and muddy water because of the similarity in the colour of the shell and the sand.

The *furcocercous cercariae* were not found in these bivalvia.

Pila virens (Lamarck) : These snails were observed in rice fields during the monsoon period. It was collected only at two locations of marshy land having an approximate area of 75 sq. foot. The density in this area was 10-15 snails per square foot. No *furcocercous* cercaria was observed in *Pila virens*.

Discussion :

To-day, the threat of introduction of schistosomiasis infection in India has increased manifold because of swiftness of modern travel and movement of population from this country to schistosome endemic areas. *Schistosoma haematobium* and *S. mansoni* are both transmitted by fresh water snails of the family *Planorbidae* in endemic areas of schistosomiasis. In the orient the hosts are operculate snails belonging to the genus *Oncomelania*. In other areas the hosts are usually *pulmonate* snails belonging to the subfamilies *Planorbinae* and *Bulininae*. In India, *F. tenuis* was reported as the vector of schistosome species in the village 'Gimvi'.

It was observed that, except for snails *F. tenuis* and *P. obesa*, no other species of snails showed the presence of *Furcocercous cercaria*. Out of the two species of snails viz., *F. tenuis* and *P. obesa*, only *F. tenuis* showed the presence of *Furcocercous cercaria* of schistosome. This conclusion was based on biometric observations of these cercaria which resembled with schistosome cercaria. These observations again confirm the findings of

earlier workers that in this area *F. tenuis* could be the only probable vector. This species breeds near human habitation near the rivulet. Its density is high during the period from October to May, when the rivulet is put to maximum use by the local inhabitants. It was possible to obtain maximum number of *furcocercous cercariae* from *F. tenuis* snails during February, March, April and May.

As will be seen from Table-II, the snail density was low during monsoon months and no *furcocercous cecariae* were observed during this period. The possibility of human contact with the rivulet was also less during this period due to heavy rainfall in the area. With the gradual increase in the snail population and recession of monsoon from the month of October onwards, the possibility of exposure of snail population to schistosome infection also increased, reaching its peak in May when the rivulet was subjected to maximum use by the local population. Incidentally, snail density was also highest during this period.

Therefore, there appears to be closer relationship between human activity, *F. tenuis* snail density and *Schistosoma* infection in this area. This will be obvious from the density pattern and presence of *furcocercous cercaria* observed during different months.

It is hoped that further studies may help us in understanding the role played by the different snail species along with other prevalent snails in the maintenance of schistosomiasis infection for such a long period.

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Table — 1

The snails observed in endemic area of schistosomiasis, village Gimvi, 1978-79

Months	<i>F. t.</i>	<i>P. o.</i>	<i>M. tub</i>	<i>I. exu.</i>	<i>Pisidium</i>
January	+	+	—	—	—
February	+	+	—	—	—
March	+	+	+	—	—
April	+	+	+	—	—
May	+	+	+	+	+
June	—	+	—	—	—
July	—	—	—	—	—
August	—	—	—	—	—
September	—	+	—	—	—
October	+	+	—	—	—
November	+	+	—	—	—
December	+	+	—	—	—

+ = Present;

— = Absent.

Table — II

The density and infection rate of snails with furcocercous cercaria in endemic areas of schistosomiasis village Gimvi, 1978-79.

Ferrisia tenuis

Months	Snail density per sq. foot of rivulet.	Infection rate of snails		%	Remarks
		Total No. of snails observed	Total No. of positive snails		
January	150	200	0		
February	275	200	2	1.0	) Furcocercous cercaria with eye spot.
March	450	300	4	1.3	
April	725	300	5	1.6	) Furcocercous cercaria with eye spot.
May	2750	400	7	1.7	
June	—	—	—	—	
July	—	—	—	—	
August	—	—	—	—	
September	—	—	—	—	
October	15	200	0	0	
November	65	200	0	0	
December	85	200	0	0	

Table — III

The density and infection rate of snails with furcocercous cercaria in endemic areas of schistosomiasis village Gimvi, 1978-79.

Paludomus obesa

Months	Snail density per sq. foot of rivulet.	Infection rate of snails			Remarks
		Total No. of snails observed	Total No. of positive snails	%	
January	65	200	1	0.5	Furcocercous cercaria without eye spot
February	85	200	0	—	—
March	105	200	0	—	—
April	75	200	0	—	—
May	55	200	0	—	—
June	22	200	0	—	—
July	—	—	—	—	—
August	—	—	—	—	—
September	13	200	0	—	—
October	22	200	0	—	—
November	45	200	2	1.0	Furcocercous cercaria without eye spot
December	55	200	4	2.0	—

TABLE — IV

The density of other snails in endemic area of schistosomiasis, village Gimvi, 1978-79. M. tuberculatus, I. exustus, Pisidium species.

Months	Snail density per square foot in rivulet			Remarks
	<i>Melanoides tuberculatus</i>	<i>Indoplanorbis exustus</i>	<i>Pisidium species</i>	
January	—	—	—	Furcocercous cercaria Not observed
February	—	—	—	
March	2	—	—	
April	3	—	—	
May	2	3	2	
June	—	—	—	
July	—	—	—	
August	—	—	—	
September	—	—	—	
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Antimalarial Activity of 'Sudarshan Ghan Vati' —An Ayurvedic Preparation

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An ayurvedic preparation 'Sudarshan Ghan Vati', was tried in malaria cases suffering from *P. vivax* malaria in Thane District Maharashtra. Following promising activity shown by this ayurvedic preparation, a trial on laboratory rodent model was undertaken. In this trial Peters 4-day test was employed to assess the efficacy of the preparation. The antimalarial activity of the formulation was confirmed.

Introduction :

A preliminary study was carried out to evaluate the antimalarial activity of an ayurvedic preparation (Sudarshan Ghan Vati), containing a number of indigenous plant ingredients, against cases of malaria. The preparation is a mixture of constituents obtained from 44 medicinal plants. In order to confirm its antimalarial activity a study was designed to evaluate the efficacy of the product in *P. berghei yoelii* — mouse model.

Material and Methods :

Maintenance of *P. berghei yoelii* strain — *P. berghei yoelii* strain was maintained by serial blood passage. The procedure was as follows : Blood was withdrawn from the heart of a mouse infected with *P. berghei yoelii* into citrate saline and was inoculated intraperitoneally into uninfected healthy mice weighing about 25 gms. After 4 days of inoculation the parasites

appeared in the peripheral blood circulation.

Preparation of Inoculum : A smear was made by snipping off a portion of the tail of the infected mouse and collecting a drop of blood on a slide. The smear was stained with 10% Giemsa stain. Parasite count was made from this smear by counting 10,000 red blood cells (rbc). Percentage of infected cells was calculated

Total rbc count of the same mouse was taken by counting the rbcs in a Neubauer chamber. On the basis of percent parasitaemia and total cell count, number of parasites per ml was calculated. The desired inoculum was prepared by diluting the cell suspension with citrated saline.

In the present study an inoculum of 1×10^6 parasites per mouse was used.

The Ayurvedic Compound : The Ayurvedic compound 'Sudarshan Ghan Vati' is a mixture of extracts from 44

medicinal plants. Its major ingredient 'Swertia chiraita' constitutes 50% of the preparation (w/w). The other ingredients taken in equal quantities constitute the remaining 50%. Following are the main ingredients :—

Santalum album *Curcuma longa*,
Cedrus deodaru, *Acorus calamus*,
Cyperus rotundus, *Terminalia chebula*,
Zingiber officinalis.

P. berghei yoelii strain obtained from National Institute of Communicable Diseases, New Delhi was used for evaluating blood schizontocidal activity of the preparation. 'Sudarshan Ghan Vati' and its major ingredient 'Swertia chiraita' were administered to groups of mice in a dose range of 10 mg — 100 mg by the oral route. The screening of the antimalarial activity was done by applying 4-day test developed by Peters.¹ In this test, drug is administered to experimental animals on day 0 (D_0) 4 hours after infection and for subsequent three days thereafter. Blood smears were taken from $D_0 + 4$ to $D_0 + 14$ ($D_0 + 4$ is 5th day of infection and $D_0 + 14$ is 15th day of infection) to record the parasitaemia. Parasite clearance from the blood was taken as an indication of efficacy of the drug.

Results :

Table I shows efficacy of the drug against clinical cases of malaria. It was found to be clinically effective in doses of 2.1 gm daily for 7 days in patients suffering from malaria. The parasites were cleared from the blood within 5 days of starting the treatment in the patients with *P. vivax* infection.

In the patients with *P. falciparum* infection parasite index noticeably reduced and in one of the patients, blood smears became negative on 5th day (Table I). Table II shows the activity of the compound 'Sudarshan Ghan Vati' in mice. The parasites were not cleared from the blood at dose levels of 10-50 mg kg^{-1} though there was a drop in parasitaemia as compared to the controls. At 100 mg kg^{-1} level the drug was found to be active. Blood smears were negative and all mice survived till the observation period of 21 days. On the other hand, control (untreated) mice showed a mean survival time of 8.8 days. These mice showed anaemia and revealed sluggish movement.

Table III shows the results of drug treatment with 'Swertia chiraita', the major component of the preparation 'Sudarshan Ghan Vati'. In this case when similar dose range was employed, there was a marked difference in the results. Parasites were cleared at doses 50 mg kg^{-1} and 100 mg kg^{-1} from only 40% mice. Only 40% mice receiving the above treatment survived the 21 day observation period.

Discussion :

The present investigation reports the evaluation of the Ayurvedic compound 'Sudarshan Ghan Vati' considered effective against blood stages of *P. vivax* & *P. falciparum* infection, in *P. berghei yoelii* mouse model. In addition to the compound, its major component 'Swertia chiraita' was also evaluated.

The study shows that 'Sudarshan Ghan Vati' demonstrates antimalarial

activity against blood stages of *P. berghei yoelii* in mouse model. The drug 'Sudarshan Ghan Vati' was effective at 100 mg kg⁻¹. However, 'Swertia chiraita', its major component, was not found as effective as the mixture, at the doses employed. When screened singly, there was only 40% survival of mice whereas, the compound showed 100% survival at the same dose level.

It can be therefore said that 'Sudarshan Ghan Vati' has antimalarial activity. Its major component 'Swertia chiraita' needs further evaluation as it has pro-

duced antimalarial action in only 40% mice at dose levels employed. As regards other ingredients, further studies are required to elucidate their antimalarial action.

Acknowledgements :

The authors are grateful to Dr. V. R. Deshpande, Director, Haffkine Institute for his support.

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Table I

Field trials of 'Sudarshan Ghan Vati' against *P. vivax* and *P. falciparum* infected patients.

Species	No. of patients	Parasite index Pre-treatment	Parasite index post-treatment		
			No. of Patients	5th day	3 weeks
<i>P. vivax</i>	7	M*	3	-ve	-
		H**	2	-ve	-
		R***	2	-ve	-
<i>P. falciparum</i>	5	M	4	Rare	-ve
			1	-ve	-

* M = Moderate : 1 parasite/field.

** H = Heavy : 3-4 parasites/field.

*** R = Rare : 1 parasite/5-6 fields.

Table II

Antimalarial activity of 'Sudarshan Ghan Vati' against *P. berghei yoelii*.

Dose mg/kg	Mean percent parasitaemia				M.S.T. in days	Percent survivals
	D ₀ + 4	D ₀ + 8	D ₀ + 12	D ₀ + 14		
10	1.5	5.5	1.0	—	8.6	—
20	2.0	5.0	—	—	9.6	—
50	-ve	2.55	—	—	8.0	—
100	-ve	-ve	-ve	-ve	14.0	100
Control	10	50	—	—	8.8	—

Table III

Antimalarial activity of 'Swertia chiraita' against *P. berghei yoelii*.

Dose mg/kg	Mean percent parasitaemia				M.S.T. in days	Percent survivals
	D ₀ + 4	D ₀ + 8	D ₀ + 12	D ₀ + 14		
10	-ve	-ve	15.0	—	11.3	—
20	-ve	3.0	8.0	—	11.0	—
50	-ve	-ve	—	—	12.0	40
100	-ve	-ve	—	—	12.0	40
Control	10	50	—	—	6.75	—

Studies on the Larvicidal Properties of *Nerium indicum* Mill (Apocynaceae), Leaves

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Nerium indicum Mill, Syn. *N. Odorum* Soland., popularly known as "Kaner" is small genus of shrubs distributed in the mediterranean region and sub-tropical Asia. *Nerium indicum* is reported to have larvicidal activity. Various extracts of *Nerium indicum* leaves were evaluated for mortality against 3rd and 4th instar mosquito larvae (*Culex p. fatigans*). It was observed that petroleum ether extract showed promising activity and hence petroleum ether extract was fractionated by using alumina column. Fraction II (benzene: methanol — 80:20) showed 100% mortality at 0.01% concentration.

Introduction :

Nerium is a small genus of shrubs distributed in the mediterranean region and sub-tropical Asia. Three species are found in India. The plants are grown as ornamental shrubs for their showy flowers. *Nerium indicum* Mill (Kaner) is frequently grown in gardens throughout India for its fragrant showy flowers. All parts of this plant are poisonous at higher doses. It is reported by Chopra *et al*¹ that the plant also possesses medicinal properties.

Alcoholic extracts of root, leaf and flower show antibacterial activity. Root extract is toxic to black carpet beetle larvae. Kerosine extract of fresh flowers is active against rice weevil, *Sitophilus oryzae* and is more active than pyrethrum extract². Leaves, stems and flowers contain a small amount of alkaloids. Pharmacological and chemical investigation of *Nerium* species is reported by Atakisnieva³. A decoction

of leaves is used to destroy germs infecting wound. Aqueous extract of leaves, branches, roots and flowers are toxic to certain insects. Basu *et al*.⁴ have reported occurrence of plumericin in *N. indicum* which is the lactonic constituent of *Plumeria rubra* which also belongs to *Apocynaceae* family.

Present paper is about mosquito larvicidal activity of various extracts and chromatographic fractions from petroleum ether extract of leaves of *Nerium indicum*. The plant material was collected from Wathar region in Satara District.

MATERIALS AND METHODS :

Nerium indicum leaves were dried in shade and then powdered to uniform mesh. One kg powdered material was extracted in Soxhlet with petroleum ether (40-60°C), ether, chloroform and methanol serially. All the above extracts were made solvent free using water bath. Different concentrations of all

Table — 1
Mosquito larvicidal activity (*Culex p. fatigans*) of different
extracts of *Nerium indicum* leaves.

No.	Extract	% Concentration	% Mortality (24 hrs.)
1.	Petroleum ether	0.08	100
		0.06	100
		0.04	100
		0.02	68
2.	Ether	0.08	48
		0.04	24
3.	Chloroform	0.08	36
		0.04	12
4.	Methanol	0.08	20
	Solvent control	2 ml acetone	Nil
	Water control	—	Nil

Table — 2
Mosquito larvicidal activity (*Culex p. fatigans*) of alumina column
chromatographic fractions of petroleum ether extract of *Nerium indicum*
leaves.

No.	Fraction	% Concentration	% Mortality (24 hrs.)
1.	Fraction I	0.02	24
		0.01	16
2.	Fraction II	0.02	100
		0.01	100
3.	Fraction III	0.02	36
		0.01	12
4.	Solvent control	2 ml acetone	Nil
5.	Water control	—	Nil

extracts were prepared in acetone and mosquito larvicidal (*Culex p. fatigans*) activity was evaluated as per method recommended by W.H.O.⁵.

The larvae (*Culex p. fatigans*) maintained by Zoonosis Department, Half-

kine Institute were used for the experiments. Solvent control and water control were also taken.

The active extract i.e., petroleum ether extract was fractionated using alumina column and eluting it further

with petroleum ether (Fraction I), benzene: methanol (80:20) (Fraction II) and methanol (Fraction III). All these fractions were tested for mosquito larvicidal activity following the method referred above.

RESULTS AND DISCUSSION :

Various concentrations of petroleum ether, ether, chloroform and methanol extracts of *Nerium indicum* (leaves) were prepared in 2 ml acetone. They were screened for mosquito larvicidal (*Culex p. fatigans*) activity. Twenty five larvae were used for each test and mortality was observed upto 24 hrs. It can be seen from Table 1, that 0.08, 0.06 and 0.04 per cent of petroleum ether extract produced 100 percent mortality whereas 0.02 percent of petroleum ether extract showed 68 per cent mortality. All other extracts at 0.08 per cent concentration did not show promising activity.

After chromatographic fractionation of petroleum ether extract of *Nerium*

indicum (leaves) and evaluating various eluates for their activity, it was observed that Fraction II showed prominent activity (Table 2). This fraction produced 100 percent mortality at 0.01 per cent concentration. Remaining eluates did not show any significant results.

From the above preliminary results it seems that *Nerium indicum* is a good source from the indigenous plants to investigate chemically pure mosquito larvicidal principle. Further work will be done to purify benzene: methanol (80 : 20) eluate (Fraction II) for chemical identification and biological evaluation.

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