spectrum of the standard band with corresponding sample band using a CAMAG TLC scanner 3. Linear relationship was obtained in the concentration range of 200-1000 ng/spot for nicotine with a correlation coefficient of 0.996. The precision of the instrument was checked by repeated scanning of same spot for seven times and % RSD was found to be 0.254. The repeatability of the method was tested by analyzing 600 ng/spot of the standard solution of nicotine after application on TLC plates (n=7). The result showed a % RSD of 2.9. Accuracy of the method was evaluated by measuring the recovery and average pecentage recovery was found to be 99.29% (Table 2).

The validated method was adopted to dtermine the content of nicotine from different brands of *Gutkha*. The contents of nicotine was found to varying from 0.1 to 0.5% w/w. The results obtained by HPTLC was compared with the results of UV spectrophotometric method. The comparative data showed that there is no significant difference in results of two methods (Table 3).

In conclusion, percentage nicotine content in different of brands of Gutkha was estimated by newly developed HPTLC method. The findings of this study indicates that all the brands of Gutkha containes considerable amount of nicotine (0.1 to 0.5% w/w).

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Antibacterial and Antifungal Activities of Spiroazetidinones

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Spiroazetidinones synthesized from the reaction of diphenylketene, generated *in situ* from thermal decomposition of azibenzil, with 3-arylimino-1-methylindol-2-ones and with 3-aryliminobornan-2-ones have been screened for antibacterial and antifungal activities. The compounds have considerable antibacterial and antifungal activities.

Azetidinones have been studied thoroughly due to their potential biological properties. Several azetidinones with antibacterial, antifungal, herbicidal, antiinflammatory and anticonvulsant activities have been reported[1]. Sulfazecin, isolated from Pseudomonas species and chromobacterium violaceum, are reported to have marked activity against Gram negative bacteria[2]. Therefore, it was

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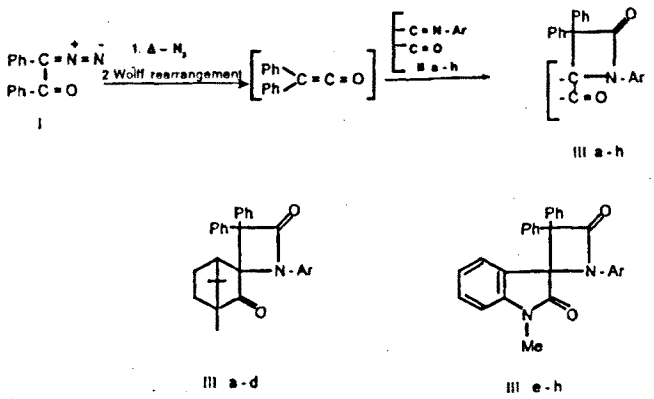
considered pertinent to study the antibacterial and antifungal activity of new spiroazetidinones[3] synthesized according to Scheme 1.



Where Ar = IIIa, e: Ph - IIIb, f; $C_6H_4CH_3$ - p. III C, g; $C_6H_4OCH_3$ - p. IIId, h; C_6H_4Cl - p.

Scheme 1 : Synthesis of Spiroazetidinones

An equimolar mixture of azibenzil (I) and 3-arylimino-bornan-2-ones (II a-d) was heated to reflux in thiophene free dry benzene (80 ml) for 6 h under a stream of nitrogen. The reaction mixture was allowed to stand overnight at room temperature, the solvent removed under reduced pressure and the residue crystallised from ethanol to give spiroazetidinones (III a-d) in 62-75% yield. 1-Aryl-1-methyl-3, 3-diphenyl-2-oxospiro (azetidin-4, 3'-indol-2-ones) (IIIe-h) were prepared in similar manner using azibenzil (I) and 3-arylimino-1-methylindol-2-ones (IIe-h).

The compounds were screened for antibacterial activity by agar plate technique[4]. Concentrations varying from 5.0 to 50.0 µg/ml of each compound were prepared by dissolving the compounds in propylene glycol. These compounds were screened against *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae*. The results are listed in Table 1.

The effect of these spiroazetidinones on growth of three fungi namely, *Cochliobolus sativus, Fusarium oxysporum* and *Citrus sinensis* was observed by food-poisoned technique[5]. Fungi were treated with five concentration of compound solution ranging from 100-500 µg/ml. Colony diameter was measured every 24 h of treatment upto 72 h. The optimum result was observed at 48 h. Experiments were set in triplicate and repeated twice. The antifungal activity was recorded in terms of percentage inhibition of colony growth (Table 2).

The results listed in Table 1 indicate that the compounds have good activity against E.coli (MIC : 50-200 µg/ml) but have moderate to poor activity against *S. aureus* and *K. Pneumoniae* (MIC : 100-200 µg/ml and 500 µg/ml, respectively). Further the compounds (IIIe-h) having indolinone moiety are more potent (MIC ranging between 50-100 µg/ml against *E. coli* and 100-200 µg/ml against *S. aureus*) than those having bornanone moiety (IIIa-d) where MIC vary between 60-200 µg/ml against *E.coli* and 150-200 µg/ml against *S. aureus*. Compounds IIIg and I'IIh inhibited growth of *K. pneumoniae* also at 500 µg/ml MIC, while no compound having bornanone

TABLE 1: ANTIBACTERIAL ACTIVITY OF SPIROAZETIDINONES

Compound No.	MIC in µg/ml against		
	E. coli	*S. aureus*	*K. pneumoniae*
IIIa	200	200	-
IIIb	100	200	-
IIIc	70	150	-
IIId	60	150	-
IIIe	100	100	-
IIIf	60	150	-
IIIg	60	120	500
IIIh	50	100	500

The control solvent was tested everytime and found to have no effect

TABLE 2: ANTIFUNGAL ACTIVITY OF SPIROAZETIDINONES HAVING BORANONE AND INDOLINE MOITIES.

Compound No.	Concentration	% Growth inhibition		
	µg/ml	C. *sativus*	F.*oxysporum*	C.*sinensis*
IIIc	100	22.40	20.28	20.18
	200	37.38	37.20	30.25
	300	50.48	45.49	34.40
	400	68.90	65.50	38.15
	500	76.43	72.14	62.35
IIId	100	25.10	22.84	20.24
	200	38.45	32.65	30.65
	300	54.24	45.56	34.32
	400	75.26	60.33	38.75
	500	80.18	74.84	50.25
IIIg	100	38.35	28.50	25.24
	200	55.45	45.95	36.64
	300	70.32	60.36	48.45
	400	80.45	75.75	65.20
	500	100.00	90.34	80.25
IIIh	100	40.42	30.60	26.37
	200	65.30	50.45	35.40
	300	80.30	70.42	60.30
	400	90.15	80.10	78.05
	500	100.00	100.00	80.00

*Colony growth was 100% in control, Mean of the three replicates

moiety could inhibit the growth of this species. In particular series, the compound having p-chloro substituent is the most potent followed by p-methoxy, p-methyl and unsubstituted phenylring.

A perusal of Table 1 and 2 reveals that all the compounds were active against the fungi tested. However, the degree of inhibiton varied both the compounds as well as with the fungus. The inhibition is varying between 37.4 to 100%. almost all the compounds have highest activity against *C. sativus* and least activity against *C. sinensis*. Here also the compounds having indolinone ring (IIIe-h) are more potent in comparison to their bornanone counterpart (IIIa-d) and in a particular class the compound having p-chloro group is the most active. At 500 µg/ml IIIg and IIIh inhibited 100% growth in case of *C. sativus*, while IIIc and IIId could inhibit only 80% and 76.5% growth at the same concentration. Compound IIIh inhibited 100% growth of *f. oxysporum* also at 500 µg/ml while IIId could inhibit only 75% growth at same concentration.

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