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THE ANTIBACTERIAL PRINCIPLES OF *SPHAERANTHUS INDICUS* ISOLATION, PURIFICATION AND ANTI-BACTERIAL ACTION

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Crude alcoholic extract of flowers of *Sphaeranthus indicus* showed antibacterial activity against 18 different species of Gram-negative and Gram-positive bacteria used. Antibacterial activity was present in alkaloidal as well as in non-alkaloid fractions. Four new alkaloids have been isolated by chromatographic methods. Broad spectrum antibacterail activity was exhibited by two isolated alkaloids.

Key words: Anti Bacterial Principles; Anti Bacterial Action.

INTRODUCTION

Sphaeranthus indicus Linn. (N.O.-Compositae) is indigenous to Indo-Pak subscontinent and is distributed throughout Pakistan. All parts of the plant find medicinal uses. The juice of the plant is styptic and diruretic. It is said to be useful in liver and gastric disorders, in jaundice, biliousness, boils, gleet, scabies, ringworm of the waist and diseases of the chest [1-4].

Kirtikar & Basu [5] reported that flowers are highly alterative, depurative, cooling and tonic. They are also used as blood purifiers in skin diseases.

Nadkarni [6] indicated that leaves of *Sphaeranthus indicus* dried in shade and powdered are useful in chronic skin diseases. It is also useful in urethral discharges and jaundice.

The object of the present study was to isolate and purify antibacterial components from flowers of *Sphaeranthus indicus.*

EXPERIMENTAL

Extraction of Sphaeranthus indicus *flowers.* Air dried flowers of *Sphaeranthus indicus* Linn. (36 kg) were percolated in EtOH for two weeks, crushed by ultraturax and filtered through Whatmann No. 1 filter paper. Crushed material was extracted with EtOH 5 times such that the last extract obtained was almost colourless. All EtOH extracts were concentrated under vacuum to brownish gummy mass. This gummy material was acidified with 10 % HCl and extracted with $CHCl_3$ to remove the non-alkaloidal portion. The acidic aqueous fraction was then basified with NH_3 and extracted thoroughly with $CHCl_3$. Evaporation of the $CHCl_3$ layer under vacuum furnished a dark brownish mass (10 mg).

Chromatography. The brownish crude mass when examined on tlc plates* showed eight spots which gave colour reactions with ceric sulphate and Dragendorff's reagent. The crude alkaloidal mass was then subjected to column chromatography for the separation and isolation of alkaloidal fractions. 10 mg of the crude alkaloidal mass was loaded on a neutral alumina (activity 3.5, 350 g, E. Merck) column and was successively eluted with different solvents in increasing order or polarity, starting with petroleum ether, chloroform and then gradually increasing the polarity to pure methanol. During the present studies four new alkaloids namely S_1, S_4, S_6 and S_7 were isolated in pure crystalline form.

Antibacterial assay system. The crude as well as purified fractions were used to determine the antibacterial activity. Five mg/ml aliquots dissolved in distilled water were used in the test.

Antibacterial activity had been tested against eighteen different species of Gram-negative and Gram-positive bacteria (Table 1). Freeze dried cultures were procured from NCTC and ATCC and were maintained on meat extract agar, stored at 40° and sub-cultures were made after 4-week intervals.

The tests were run in triplicate. Petri plates (10 x 10 cm) were prepared with trypticase soy agar (Bhakuni 7). 0.1 ml of the diluted overnight culture was poured and spread on each plate and the plates were dried for 30 min at 37°. Wells of 6 mm dia were cut with a sterile cork

*TLC plates (DC-Mikrokarteu SIF-5 x 10 cm Riedel-D-Haen AK tiengessel- Schaft. Seelze Hannover)- Solvent system $CHCl_3$:MeOH (1:3) w/w E. Merck.

borer in the inoculated agar. The wells were filled with the plant extract. 50 % ethanol in water was used as control.

The plates were incubated for 24 hr at 37°. At the end of the incubation period the inhibition zones were measured to the nearest mm. (Tables 1-2). The antibacterial activity of the crude extract was also compared with the commercial antibiotics by sensitivity disc method. (Table 3).

Sensitivity disc method. The oxoid Multodisc was used in the study. The trypticase soy agar plates were seeded with overnight culture of the test organism. An excess of inocultum was removed. Multodisc was aseptically placed over the agar and incubated at 37° for 24 hr. The zones of inhibition were measured to nearest mm. (Table 3).

RESULTS

Antibacterial studies with crude extract of flowers of *Sphaeranthus indicus* exhibited broad spectrum activity against all 18 bacterial strains used in the study (Table 1 and 2). The bioautographic studies of the crude extract also confirmed the presence of biologically active components.

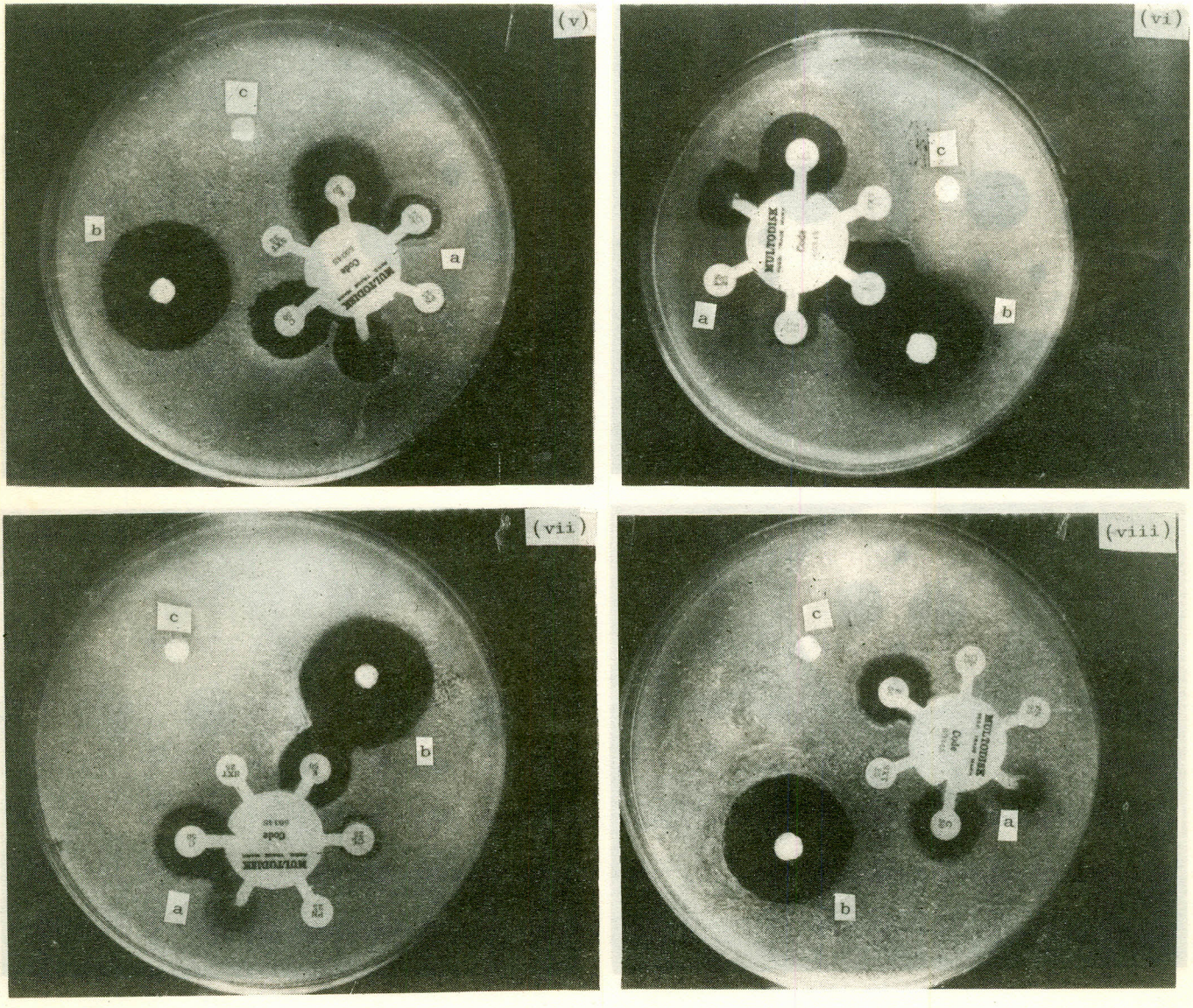

Plate I. Comparision of growth inhibition by crude ethanolic extract of *Sphaeranthus indicus* and commercial antibiotics using disc method. (v) *Staphylococcus citreus* (vi) *Staphylococcus aureus* (vii) *Staphylococcus albus* (viii) *Streptococcus pyogenes* (a) antibiotic disc (b) crude extract disc and (c) control.

A comparison of crude ethanolic extract with commercial antibiotics indicated that crude extract of flowers of *Sphaeranthus* was much more active than the antibiotic used (Table 3, Plate I).

The crude extract was fractionated further into partially purified aliquots containing alkaloidal and non-alkaloidal fractions. The results summarized in Table 2, Plates II, III, indicate the presence of activity in both fractions, but more prominent in non-alkaloidal ones. The alkaloidal fraction was further fractionated by chromatographic methods and four previously unknown alkaloids S_1, S_4, S_6 and S_7 were detected out of which two S_1 and S_6 showed antibacterial activity (Table 2, Plates II and III).

DISCUSSION

Sphaeranthus indicus is known to possess powerful medicinal properties [1, 4, 5, 6]. Its medicinal usage has attracted attention of various workers to study the chemical constituents of the plant [8 – 14]. In the present study an attempt was made to isolate and characterize biologically active components from flowers of *Sphaeranthus indicus*.

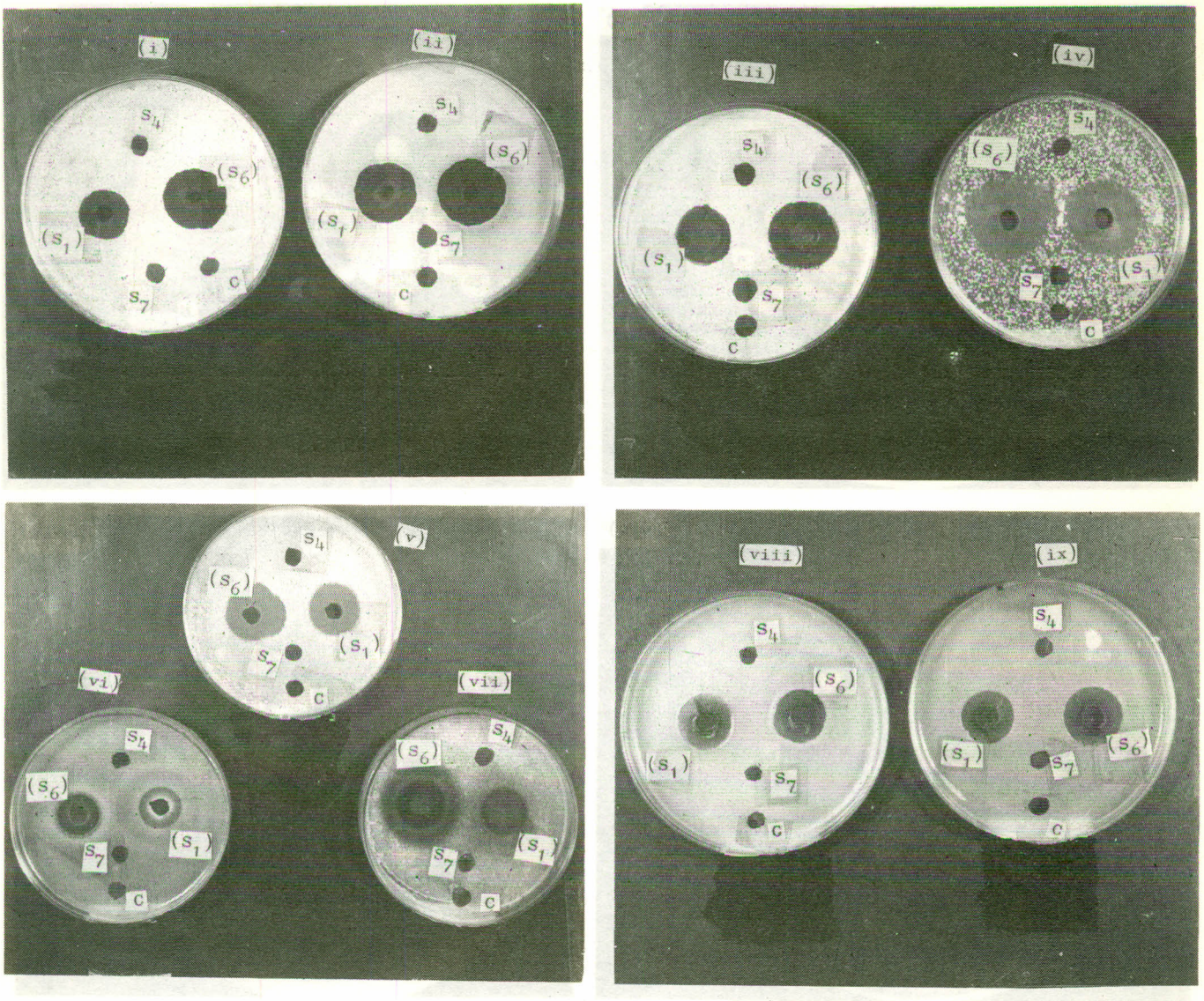

Plate II. Zone of inhibition of growth of (i) *Bacillus subtilis* (ii) *Bacillus megaterium* (iii) *Sarcina lutea* (iv) *Micrococcus lysodenticus* (v) *Staphylococcus citreus* (vi) *Staphylococcus aureus* (vii) *Staphylococcus albus* (viii) *Streptococcus pyogenes* (ix) *Streptococcus faecalis* caused by pure alkaloidal fraction (S_1), (S_4), (S_6), (S_7) isolated from *Sphaeranthus indicus* and C = control.

Table 1. Antibacterial activity of the crude extract, and major components of *Sphaeranthus indicus*.

Organism	Zone of inhibition (in mm)			
	Crude extract	Alkaloidal fraction	Non-alkaloidal fraction	Control
Bacillus subtilis	16	16	20	–
B. megaterium	18	16	20	–
Sarcine lutea	22	15	20	–
Micrococcus lysodeikticus	24	15	18	–
Staphylococcus citreus	16	16	20	–
S. aureus	18	15	20	–
S. albus	17	15	20	–
Streptococcus pyogenes	15	15	18	–
S. faecalis	18	16	18	–
Salmonella typhi	20	16	20	–
S. paratyphi A	20	12	22	–
S. paratyphi B	18	10	20	–
Shigella shigi	16	16	20	–
S. flexneri	15	12	20	–
S. sonnei	15	10	22	–
Escherichia coli	14	10	22	–
Proteus vulgaris	14	10	22	–
Pseudomonas aeruginosa	12	10	10	–

Table 2. Antibacterial activity of pure aklaloidal fractions of *Sphaeranthus indicus* (on Agar Plates)

Name of organism	Zone of inhibition (in mm)				
	S_1*	S_4*	S_6*	S_7*	Control
Bacillus subtilis	6	–	8	–	–
B. megaterium	8	–	10	–	–
Sarcina lutea	8	–	10	–	–
Micrococcus lysodeikticus	10	–	15	–	–
Staphylococcus citreus	10	–	10	–	–
S. aureus	8	–	8	–	–
S. albus	8	–	8	–	–
Streptococcus pyogenes	10	–	10	–	–
S. faecalis	10	–	10	–	–
Salmonella typhi	8	–	12	–	–
S. paratyphi A	8	–	10	–	–
S. paratyphi B	8	–	12	–	–
Shigella shigi	10	–	15	–	–
S. flexneri	10	–	12	–	–
S. sonnei	10	–	10	–	–
Escherichia coli	15	–	18	–	–
Proteus vulgaris	10	–	15	–	–
Pseudomonas aeruginosa	8	–	8	–	–

*Pure alkaloids designated as S_1, S_4, S_6 and S_7.

Table 3. Comparison of antibacterial activity of crude extract of *Sphaeranthus indicus* and commercial antibiotics***

Organism	Zone of inhibition on agar plates**							
	Crude extract disc, 50 mcg	TE 50 mcg	C 50 mcg	E 50 mcg	CL 25 mcg	PN 25 mcg	SXT 25 mcg	Control*
Bacillus subtilis	16	8	10	15	6	–	–	–
B. megaterium	18	8	10	15	5	–	–	–
Sarcina lutea	22	10	12	16	5	–	–	–
Micrococcus lysodeikticus	24	–	8	8	–	–	–	–
Staphylococcus citreus	16	8	10	15	4	–	–	–
S. aureus	18	7	10	10	4	–	–	–
S. albus	17	4	10	8	2	–	–	–
Streptococcus pyogenes	15	5	10	10	–	–	–	–
S. faecalis	18	10	12	14	6	–	–	–
Salmonella typhi	20	8	12	10	10	–	–	–
S. para typhi A	20	8	14	15	7	–	–	–
S. para typhi B	18	8	8	12	5	–	–	–
Shigella shigi	16	5	12	8	5	–	–	–
S. flexneri	15	5	10	16	4	–	–	–
S. sonnei	15	4	12	8	–	–	–	–
Escherichia coli	14	6	8	12	10	–	–	–
Proteus vulgaris	14	8	8	10	10	–	–	–
Pseudomonas aeruginosa	12	5	8	8	8	–	–	–

*Control. **Zone of inhibition in mm.
***TE (Balkacycline), C: (Balkamycin), E: (Erythrolate), CL: (Ultrasporin), PN: (Ultracillin), SXT: (Balkatrin).

The results obtained with crude extract of the flowers confirmed the previous findings of Dhar *et al.* [15]. They had reported that crude extracts of the plant had antibacterial activity against Gram-negative and Gram-positive bacteria. Results of bioautographic studies were in conformity with the results obtained by cylinder plate methods [16].

A comparison of the extract of flowers of *Sphaeranthus indicus* with commercial antibiotics indicated that the crude extract was much more active than the antibiotics. The reason was probably that the crude extract was a mixture of a number of active components as confirmed by further chromatographic methods in which initially two major components, alkaloidal and non-alkaloidal, were

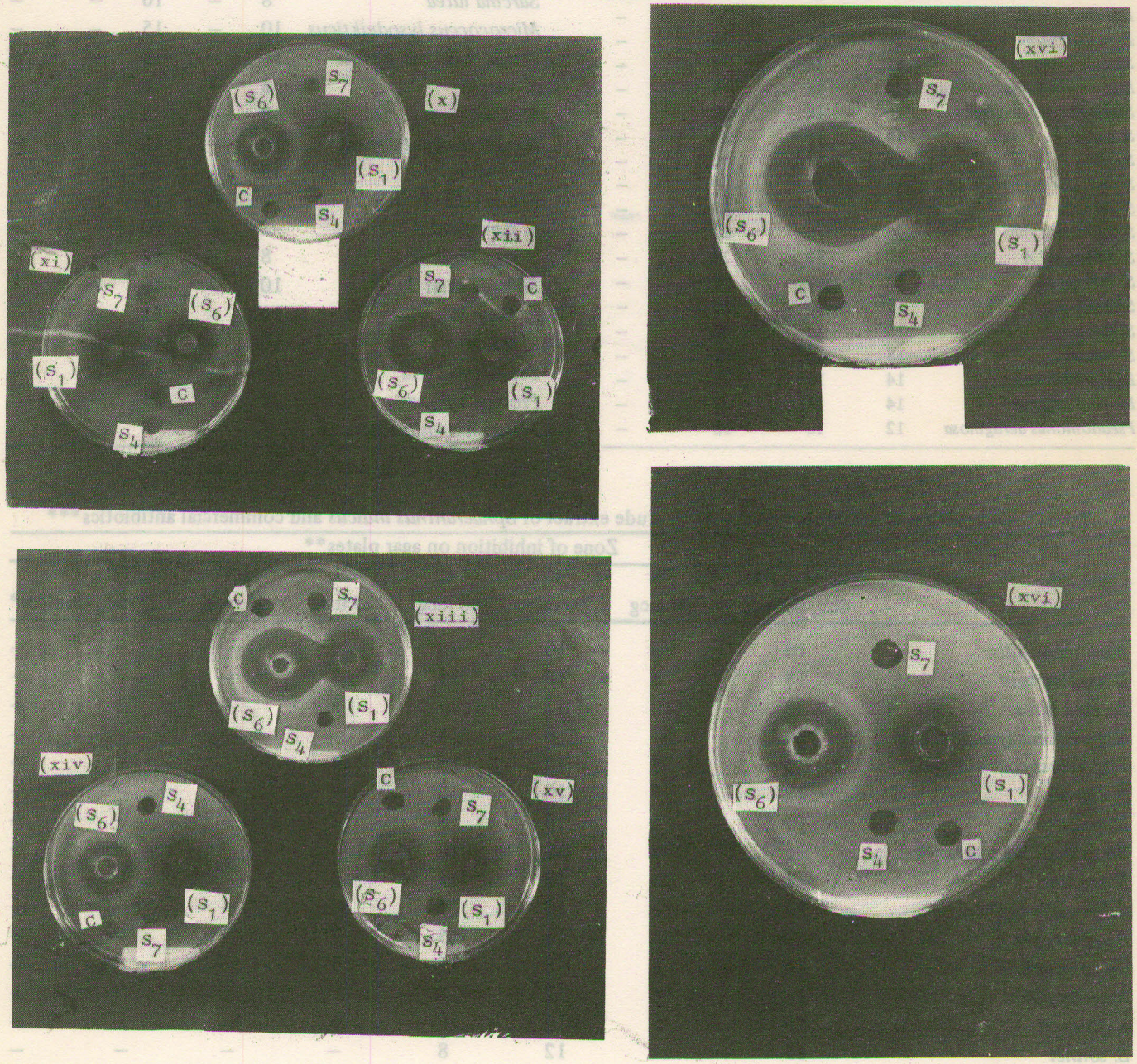

Plate III. Zone of inhibition of growth of (x) *Salmonella typhi* (xi) *Salmonella typhi para* A (xii) *Salmonella typhi para* B (xiii) *Shigella shigi* (xiv) *Shigella flexneri* (xv) *Shigella soni* (xvi) *Escherichia coli* (xvii) *Proteus vulgaris*, caused by pure alkaloidal fraction (S_1), (S_4), (S_6), (S_7) isolated from *Sphaeranthus indicus* and C = control.

separated, both possessing antibacterial activity. As indicated in Table 2 the inhibition caused by the non-alkaloidal portion was slightly more than the alkaloidal portion. It may be due to the presence of β-sitosterol which possess antibacterial activity as reported by Gupta *et al.* [17]. This substance was isolated from non-alkaloidal portion by Tiwari [13].

Further fractionation of alkaloidal fraction revealed the presence of four alkaloids S_1, S_4, S_6 and S_7 among which S_1 and S_6 were active. The presence of an alkaloid, *sphaeranthine*, molecular formula $C_{13}H_{19}NO_5$, was reported by Basu and Lamsal in 1946 [8]. The antibacterial activity of the component was not reported. The present study seems to be the first report in scientific literature in which four new alkaloids S_1, S_4, S_6 and S_7 have been reported from flowers of *Sphaeranthus indicus* among which two alkaloids S_1 and S_6 are biologically active. The details of spectrophotometric studies to be published in near future indicate that they were different from sphaeranthine reported earlier [8].

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