APPENDIX I

PLANTA MEDICA

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SURVEY OF FERNS IN GUJARAT STATE (INDIA) FOR PRESENCE OF ANTIBACTERIAL SUBSTANCES OF FERNS

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Introduction

Plants and their products appear to have been used in the treatment of infectious diseases from very long time, even before the discovery of micro-organism (Abraham, 1949). In the field of modern medical practice Mosse (1852) was the first to suggest the use of micro-organisms for therapeutic purposes. It was however, Tyndall, a physicist, who described the antagonistic action of a species of *Penicillium* to bacterial growth. Though he failed to recognise the probable cause of the phenomenon, Tyndall (1876) was the first to demonstrate the antibiotic action of a fungus. This was immediately followed by the first clear description of bacterial antagonism by Pasteur and Joubert (1877). The antibiotic research gathered momentum until it culminated in the discovery of penicillin (Chain et al. 1940) as belonging to a rare class of drugs, the systematic chemotherapeutic agents.

The systematic investigation of higher plants for antibiotic substances was revived when Osborn (1943) reported his now classical studies on antimicrobial substances from green plants. He made an experimental survey on 2300 species of higher plants belonging to 166 different families and showed that several species in the *Ranunculaceae, Cruciferae, Compositae* and *Liliaceae* contained substances that inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. Since then voluminous literature has accumulated on this subject (Nickell, 1959). Almost all plants tested, however, have been spermatophytes (Lucas and Lewis, 1944; Sander et al., 1945; Hayes, 1947; Pates and Madson, 1955; Dhar et al., 1968) and very little attention is paid to the lower vascular plants, the Pteridophytes. Maruzzella's (1961) screening of 34 ferns for antimicrobial substances is the only work exclusively on lower vascular plants. He reported extraction of antibacterial substance from 33 of the 34 specimens tested.

The present work is concerned with the examination of ferns collected from different parts of Gujarat for antibacterial contents, if any. Few species of ferns were collected from Mount Abu region in Rajasthan and they too are likewise tested.

Experimental

Methodology

All the plants were fresh, green specimens selected at random. Collections were made at different times all round the year. The fronds were thoroughly washed with water, rinsed with distilled water and then air-dried for a week. Dried fronds were mechanically ground to a powder. The pulverised powder was next extracted with methanol for 48 hours on a rotatary shaker. The extract was filtered and the filtrate concentrated under reduced pressure (bath temp. 50° C) to a desired volume.

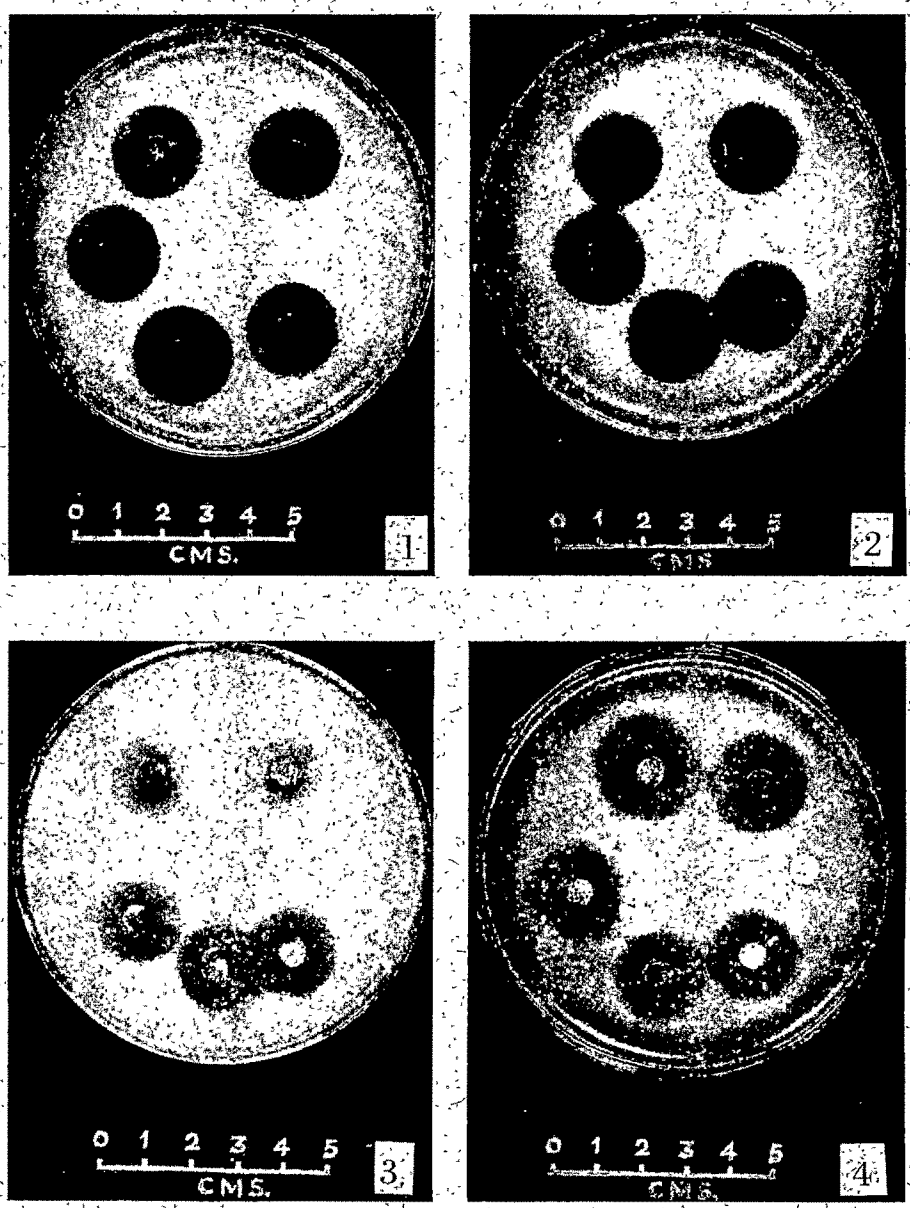
Antibacterial activity was determined by the use of impregnated filter-paper disks. The disks, 6.35 mm. in diameter and punched from No. 1 Whatman filter-paper were sterilized by dry heat at 150° C for an hour in batches in screw-capped bottles. Petri-plates were prepared the day previous to the actual testing of samples by adding 22 ml of nutrient agar to sterile petri-dishes (10 cm in diameter). The latter were then kept at 37° C for 24 hours before being overlaid with 3 ml of seed agar (1%) inoculated with the test organism. Previously sterilized filter-paper disks were impregnated with concentrated extracts and then allowed to dry at room temperature. The dried disks were placed on the seeded agar plates. A control disk impregnated with solvent only and dried was also included. After incubation at 37° C for 24 hours zones of inhibition were measured. The area of inhibition measured included that of the disk as well as surrounding zone.

Results and Discussion

The table 1 gives the list of ferns screened against the different bacterial cultures.

Table 1 and Figures 1 to 4 clearly showed that some of the lower vascular plants tested contained active antibacterial principles. This active principle or principles varied greatly in their potency from species to species. Even within the same species, the potency was found to vary not only with the stage of development but also with the age of the plant. For in most cases, the activity was observed when the plants were fertile and furthermore, the older plants revealed more pronounced activity than the young ones.

The present list of ferns examined included three species (marked with * in Table I) previously studied by Maruzzella (1961). In most instances particularly with *Nephrolepis exaltata* and *Pteris vittata* the findings of Maruzzella have been corroborated; while in case of *Adiantum trapiziforme* different results have been obtained. Whereas we obtained positive results with *Adiantum trapiziforme* against *Staphylococcus aureus* and *Bacillus megatherium*, Maruzzella reported positive results only against *E. coli*. This, however, is not unexpected since the phyto-constituents are known to vary depending on ecological factors such as climate, habitat, temperature under which the plant was growing at the time of collection as well as the differences in strains of micro-organisms tested. Regularity of occurrence under identifical conditions was established in most of the plants examined in the present studies.



Figures 1–4

Activity of *Adiantum trapiziforme against Bacillus megatherium* (Fig. 1) and *Staphylococcus aureus* (Fig. 2).

Activity of *Aleuritopteris farinosa* against *B. megatherium* (Fig. 3) and *S. aureus* (Fig. 4).

As these active antibacterial substances are capable of inhibiting the growth of particular strains of bacteria alone, it seemed that these substances might be quite specific in their mode of action. Selective inhibition of bacterial cultures used during screening by active principle from some plants is very much suggestive of their species specific nature.

All the above ferns were further tested for the presence of alkaloids. The procedure followed for extraction of alkaloids was similar to that described by Abisch and Reichstein (1960) and modified by Worthen et al. (1965). No alkaloids were, however, detected in any of the ferns examined; for the plant extracts gave negative results with Mayer's and Dragendorff's reagents.

Table I

Activity of methanol extracts of ferns against various bacteria measured by paper disc method.

Name of the plant	Activity against				
	Bacillus subtilis NCTC 8236	*Bacillus cereus* NCTC 9946	*Bacillus megatherium*	*Staphylococcus aureus* B-43-5	*Escherichia coli* B-3-2
1. *Adiantum incisum* Forsk.	–	–	–	–	–
2. *Adiantum lunulatum* Burm.	–	–	–	–	–
3. *Adiantum trapiziforme* Linn.	–	–	+++	++	–
4. *Aleuritopteris farinosa* Forsk. Fee.	–	–	+++	+++	–
5. *Athyrium falcatum* Bedd.	–	–	–	–	–
6. *Athyrium filix-femina* (L.) Roth.	–	–	–	–	–
7. *Nephrolepis cordifolia* (L.) Presl	–	–	–	–	–
8. *Nephrolepis exaltata* (L.) Schott.	–	–	–	–	–
9. *Nephrolepsis* sp. (Cultivar)	–	–	–	–	–
10. *Dryopteris crenata* (Forsk.) O. Ktze	–	–	–	–	–
11. *Pteris vittata* Linn.	–	++	–	++	–
12. *Tectaria macrodanta* (Fee.) C. Chr.	–	–	–	–	–
13. *Actinopteris australis* (Linn. f.) Link	–	–	–	–	–

– no zone of inhibition
+ 0 to 5 mm. zone of inhibition
++ 5 to 10 mm. zone of inhibition
+++ 10 to 15 mm. zone of inhibition
++++ 15 to 20 mm. zone of inhibition

Summary

13 species of Ferns available in Gujarat State (India) are examined for the presence of antibacterial substances. Methanol extracts were made of plants at different stages of growth and also of plants of different ages.

Antibacterial activity was observed in few of the ferns screened. The activity was more pronounced in old plants than in young ones. The fertile plants showed greater activity than vegetative ones.

A preliminary study for the presence of alkaloids in fern species was negative.

Acknowledgements

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