

Antimicrobial Screening of some Selected Tribal Medicinal Plants from Sriharikota Island, Andhra Pradesh, India

¹R. Bharath Kumar and ²B. Suryanarayana

¹Associate Professor, Dept. of Biotechnology,
Vignan's Engg. College, Vadlamudi, Guntur-522213

²Associate Professor (Retd), #8-307, Karnakamma Street,
Venkatagiri Town-524132

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Abstract

Sriharikota Island is important because of its rich vegetational diversity and for the fact that it is the site of a Rocket Launching Station built by the Indian Space Research Organization (ISRO). An aboriginal tribe called 'Yanadi' dwells in a few tribal pockets on Sriharikota Island. However, a few aged tribal men are still able to furnish information regarding their traditional practices of medicines and recipes. There is an urgent need to conserve their herbal wealth and preserve their traditional knowledge for the benefit of modern society.

It is with this background, along with the need to give a primary scientific basis to the tribal medicinal lore of Sriharikota Island, that a preliminary screening of antimicrobial (antibacterial and antifungal) activity screening was conducted for 81 tribal medicinal samples based on their medicinal importance and endemism/rare occurrence on the island.

Of these 81 samples, 28 have shown positive response for antimicrobial tests. Among these 8 species, namely *Alangium salvifolium*, *Aristolochia indica*, *Centropogon colocyntis*, *Datura stramonium*, *Ficus religiosa*, *Holoptelia integrifolia*, *Lawsonia innermis* and *Ventilago madaraspatana* have shown congruible immunity zone of inhibition (i.e. above 20 mm). All the data is recorded in this paper. The results are mostly in conformity of the medicinal uses and they are discussed in detail in this article.

Introduction

Yanadies are an aboriginal tribe living on Sriharikota Island in Andhra Pradesh, even after the establishment of the Rocket Launching Station. They are said to have been originally from the Malaya Peninsula, Africa or Australia.

Until the establishment of the SHAR Centre at Sriharikota, Yanadies lived in forests and near the sea coasts in traditional ways and were in co-existence with the environment. In spite of the community having drifted from the natural way of life due to the introduction of agro-rural developmental activities, a few aged tribal men are still able to furnish 'ethno-medicinal' information pertaining to their traditional practices and healings. With the help of local tribal men, 300 ethno-medicinal plants were collected together with ample information relating to healing

practices. The pharmaceutical uses of these plants are considered here under 16 categories (Table 1).

In order to give a primary scientific basis to the tribal medicinal lore of Sriharikota Island, a preliminary screening of Antimicrobial (antibacterial and antifungal) activity was conducted for 81 plant samples (such as root, root bark, stem bark, leaf, fruit, seed and whole plant etc.) chosen from the above mentioned 300 plantspecies. Twenty-eight samples showed positive responses (producing of an inhibition zone) for Antimicrobial screening tests.

Among the 28 samples, 8 species, namely *Alangium salvifolium*, *Aristolochia indica*, *Citrullus colocynthis*, *Datura stramonium*, *Ficus religiosa*, *Holoptelia integrifolia*, *Lawsonia innermis* and *Ventilago madaraspatana*, have shown a congizable immunity zone of inhibition (i.e. above 20 mm.). The results are discussed in this article.

Topography

Sriharikota Island is located at 80⁰ 21' E and 13⁰ 22' to 14⁰ N. It is a spindle shaped land mass sandwiched between the Bay of Bengal on the east and Pulicatlake on the west. It is 18 km east of Sullurpet, the nearest Railway Station connecting Chennai-Kolkata trunk line. Chennai is 98 km away from Sriharikota.

Materials and Methods

An intensive three year medico-ethnobotanical survey was conducted on Sriharikota Island from 1996-1999. Field tours at regular intervals were conducted covering all the seasons so as not to miss seasonal elements having pharmaceutical value. Plant species selected for Antimicrobial screening are used in ethnomedicine by the tribals for curing their general ailments such as infected wounds, itches, scabies, dandruff, blood motions, jaundice, fungal oral thrush and skin diseases etc. (Table 1).

Antimicrobial screening was done by the standard procedures described by Bhakuni et.al (1969,1971,1988,1990), Ikram & Inamul (1980), Aldesanmi (1988),Mbah (1993) and Ahmad et.al (1994), etc.

Preparation of Plant Extracts

The plant parts (Leaf, Rt, Rt.b, St., St.b., Wp. etc.) were washed with water, chopped into small fragments and shade dried. The dried samples were ground to powder (i.e. 20 gr. each); they were separately extracted in 200 ml methanol and acetone solvents with soxhlet apparatus. They were carefully collected into conical flasks and properly evaporated by placing them on hot water bath. Later on this, these powdered samples were carefully packed in small bottles and stored at room temperature.

Extracts of plant samples were prepared by taking 2g of dried powdered plant material in 5 ml methanol and acetone concentrations in small bottles. Filter paper discs were prepared with Whatmann No.1 filter paper discs of 6 mm diameter and they were autoclaved by keeping in a clean and dried Petri plate. The filter paper discs soaked in plant extracts were taken as test material and dried. The filter paper discs were carefully transferred to spread on cultured Petri plates. Filter paper discs were immersed in methanol and acetone also prepared and used as a control.

Media & Microorganisms

The suitable culture media was prepared by dissolving the below mentioned ingredients for the respective microorganisms. The contents were autoclaved at 15 lbs for 15 min. Microorganisms are taken as test organisms both Gram +ve, Gram-ve bacterial species and fungal sp. for antibacterial and antifungal activities of plant extracts.

Medium for bacterial sp.: **Nutrient Broth/Nutrient Agar Medium (NBM/NAM)** ingredients:

| | |
|------------------------|------------|
| Peptone | -- 10 gr |
| Beaf extract | -- 10 gr |
| Nacl | -- 5 gr |
| Dist. H ₂ O | -- 1000 ml |
| Agar | -- 15 gr |
| pH | -- 7 |

Medium for fungal sp.: **Sabaroud Broth/ Sabaroud Agar Medium (SBM/SAM)** ingredients:

| | |
|------------------------|-----------|
| Glucose | -- 40 gr |
| Peptone | -- 10 gr |
| Agar | -- 10 gr |
| Dist. H ₂ O | --1000 ml |
| pH | -- 6 |

Microorganisms:

Bacteria:

Gram +ve:

Staphylococcus aureus - This major pathogenic sp. cause boils, wound infections and food poisoning in humans.

Micrococcus sp. – Harmless saprophyte occurs in soil and fresh water and also found on the skin of humans and animals.

Gram –ve:

Salmonella tiphymurium- Pathogenic for humans causing enteric fevers (such as Typhoid and Paratyphoid fevers).

Escherichia coli – Occurs in the lower portion of intestine of humans and warm blooded animals –causes gastroenteritis, urinary tract infection.

Pseudomonas aeruginosa – Pathogenic for humans and animals. Isolated from wounds, burns and urinary tract infections.

Fungi:

Pathogenic fungi:

Candida albicans – Pathogenic, causing **cadidiasis** -a disease of the mucus, vagina and alimentary tract infections.

The above mentioned microbial cultures were maintained on their respective media in slants at 4⁰C. (as per

standard procedures).

Preparation of Test Plates & Antimicrobial Testing:

The agar test plates (Petri dishes) were prepared by pouring about 15 ml of the medium. These test plates were placed under aseptic conditions at 4⁰C for 24 hours to control sterility. After solidifying the media (NAM and SAB), the inoculums (bacteria 24 hrs and fungi 48 hrs.) homogenous suspension was uniformly spread on their respective test plates. The filter paper discs were prepared in methanol (M) and acetone (A) extracts as taken for control.

The filter paper discs were carefully placed the spreaded culture test plates and incubated at appropriate temperature for bacteria at 37⁰C for 24 hrs. and fungi 30⁰C for 48 hrs. After the incubation period, the test plates were examined for inhibitory zones. The diameters of inhibitory zones were recorded (Table 2). All determinants were made at least in triplicate for each of the test organisms in different extracts, and recorded.

Results & Discussion:

Sixteen field tours of 5-7 days duration for each tour were conducted for three years during 1996-1999, with the assistance of local tribal men. Three hundred species having medicinal uses plus ample field notes were collected, including flowering, fruiting season, flower colour and other phenological data.

A total of 81 methanol and acetone soxhlet extracts from 77 plant species popularly used by the tribals as herbal remedies were screened in, in-vitro studies to evaluate their antibacterial and antifungal activity. Twenty eight samples exhibited positive activity (Table 2). Of these 28 samples, 8 species exhibited a cognizable inhibitory zone (> 20 mm.), i.e. 10 % of the total test samples (81 plant extracts), Table 4.

The inhibitory activity of methanol extracts is comparatively more than those of acetone extracts. The methanol and acetone extracts had high inhibitory activity in Gram +ve bacteria and comparatively less in both Gram -ve bacteria and in the pathogenic fungus, *Candida albicans*.

Popular medicinal plants useful to tribals selected based on therapeutic values and screened in vitro for antimicrobial activity of acetone and methanol plant extracts was observed on five strains of two gram-positive, three gram-negative bacteria and one pathogenic fungi. The plant species were selected based on their tribal medicinal importance, and corresponding pharmaceutical categories are given in Table 1.

Table 1. Classification of Selected Tribal Medicinal Plants of Antimicrobial Analysis.

| <u>CATEGORY</u> | <u>PHARMACEUTICAL CATEGORY</u> | <u>No.of Sps.</u> |
|-----------------|------------------------------------|-------------------|
| No. | | |
| 1. | Respiratory and bronchial problems | 4 |
| 2. | Cordiac | 1 |
| 3. | Urinary and kidney | ¾ |
| 4. | Piles and constipation | ¾ |
| 5. | Arthritis and rheumatism | 22 |
| 6. | Anodyne elements | 1 |
| 7. | Sexual problems | 3 |

| | | |
|-----|---|---|
| 8. | Skin and dermatitis | 9 |
| 9. | Ophthalmic and E.N.T | 7 |
| 10. | Psychoactive and nervous disorders | 1 |
| 11. | Jaundice and liver disorders | 1 |
| 12. | Digestive and stomachic | 4 |
| 13. | Cooling and allterative | 1 |
| 14. | Antipyretic, anthelmintic and antiperiodic | 2 |
| 15. | Antidotes, antivenom (scorpion sting and Snake bite) | 2 |
| 16. | Diabetes | 3 |

Total = 61

The samples that were selected based on their therapeutic uses, fall more or less under all 16 pharmaceutical categories. Out of these 28 samples which have shown positive samples selected based on their therapeutic uses, among these 16 pharmaceutical categories, antimicrobial results were exhibited in the form of immunity zone, fall under 11 pharmaceutical categories only. Further 7 samples of antifungal 22 samples of antibacterial in the 3 samples have exhibited both antifungal and antibacterial inhibition.

Further immunity response for PC - 5, was observed in 7 species and for PC - 8 is observed in 8 species, and immunity response against other PC. categories are only seen 1 or 2 species. (Table 3).

Table-3. Summary Of Antimicrobial Screening.

| S.No. (1) | Plant extract sample PC.No. (2) | plant part (3) | <u>Microbial test elements.</u> | | |
|--|---------------------------------------|----------------------|---------------------------------|---------------|-----------|
| | | | (G+ve) (4) | (G+ve) (5) | F. (6) |
| Respiratory and bronchial : PC - 1. | | | | | |
| 1. | <i>Centella asiatica</i> | Lf. | + | - | - |
| 2. | <i>Datura stramonium</i> | Sd. | + | - | - |
| 3. | <i>Hybanthus enneaspermus</i> | Lf. | + | - | - |
| 4. | <i>Sarcostemma acidum</i> | St. | - | - | + |
| Arthritis & rheumatism: | | | | | |
| PC - 5. | | | | | |
| 5. | <i>Alangium salivifolium</i> | Lf. | - | - | + |
| 6. | <i>Caesalpinia bonduc</i> | Sd. | - | + | - |
| 7. | <i>Citrullus colocynthis</i> | Lf. | - | - | + |
| 8. | <i>Ficus religiosa</i> | St.b | + | - | - |
| 9. | <i>Garcinia spicata</i> | Rt.b | + | - | - |

| | | | | | |
|---------------------------|--------------------------------|-------|---|---|---|
| 10. | <i>Holoptelia integrifolia</i> | St.b | + | - | + |
| 11. | <i>Toddalia asiatica</i> | Rt. | + | - | - |
| Anodyne elements : | | | | | |
| PC - 6. | | | | | |
| 12. | <i>Ficus religiosa</i> | St.b. | + | - | - |

| | | | | | |
|-----|-----|-----|-----|-----|-----|
| (1) | (2) | (3) | (4) | (5) | (6) |
|-----|-----|-----|-----|-----|-----|

Skin and dermatitis :

PC - 8.:

| | | | | | |
|-----|-------------------------------|------|---|---|---|
| 13. | <i>Crataeva magna</i> | Lf. | + | - | - |
| 14. | <i>Cissus vitifolia</i> | Rt. | + | - | - |
| 15. | <i>Datura stramonium</i> | Lf. | + | - | - |
| 16. | <i>Eugenia bracteata</i> | Lf. | + | - | - |
| 17. | <i>Ficus religiosa</i> | Lf. | - | - | + |
| 18. | <i>Gmelina asiatica</i> | Fr. | + | - | - |
| 19. | <i>Lawsonia inermis</i> | Lf. | + | - | - |
| 20. | <i>Ventilago madraspatana</i> | Rt.b | + | - | - |

Ophthalmic and ENT :

PC - 9.:

| | | | | | |
|-----|---------------------------|-----|---|---|---|
| 21. | <i>Eugenia bracteata</i> | Rt. | + | - | - |
| 22. | <i>Lawsonia inermis</i> | Lf. | + | - | - |
| 23. | <i>Sarcostemma acidum</i> | St. | - | - | + |

**Psychoactive and
nervous disorders :**

PC - 10:

| | | | | | |
|-----|--------------------------|-----|---|---|---|
| 24. | <i>Centella asiatica</i> | Lf. | + | - | - |
| 25. | <i>Datura stramonium</i> | Sd. | + | - | - |

**Jaundice and Liver
disorders: PC - 11.**

| | | | | | |
|-----|---------------------------|-------|---|---|---|
| 26. | <i>Phyllanthus amarus</i> | W.pl. | + | - | + |
|-----|---------------------------|-------|---|---|---|

| | | | | | |
|-----|-----|-----|-----|-----|-----|
| (1) | (2) | (3) | (4) | (5) | (6) |
|-----|-----|-----|-----|-----|-----|

Digestive and stomachic :

PC - 12:

| | | | | | |
|-----|----------------------------|-------|---|---|---|
| 27. | <i>Aristolochia indica</i> | Rt. | + | - | - |
| 28. | <i>Mangifera indica</i> | St. | + | - | - |
| 29. | <i>Mimusops elengi</i> | St.b. | + | - | - |

Cooling and alterative :

PC - 13:

| | | | | | |
|-----|---------------------------|-----|---|---|---|
| 30. | <i>Colubrina asiatica</i> | Lf. | - | + | + |
|-----|---------------------------|-----|---|---|---|

Antidotes and antivenom:

PC - 15 :

| | | | | | |
|-----|----------------------------|-----|---|---|---|
| 31. | <i>Aristolochia indica</i> | Rt. | - | + | + |
| 32. | <i>Caesalpinia bonduc</i> | Sd. | - | + | - |

Diabetes : PC - 16.

| | | | | | |
|-----|---------------------------|-----|---|---|---|
| 33. | <i>Casearia esculenta</i> | Rt. | - | + | - |
|-----|---------------------------|-----|---|---|---|

Eighty-one samples when subjected to inhibition activity test 28 samples, are positive for inhibition; rest of the 54 samples are passive. Details are as follows:

Immunity response of species against microbial activity as per pharmaceutical categories is as follows:

PC 8 : 8 sps ; PC 5 : 7 sps ; PC 1 : 4 sps ; PC 9 & 12 : 3 sps ;

PC 10 & 15 : 2 sps ; PC 6, 11, 13 & 16 : 1 sps.

Upon critical study and to summarize the antimicrobial activity test, it is noticed that out of 28 samples with positive response the congeizable inhibition could be observed i.e. above 20 mm in 8 species only 6 species have exhibited immunity zone against G+ve bacteria. Expression for inhibition is either for both test elements vig. bacteria and fungus or one element ie. bacteria or fungus the solvent extracts in A/M (Acetone/ Methanol) [Table 4].

Table 4. Above 20 mm Inhibition.

| S.No. | Plant name | part used | G- | G+ | F. |
|-------|------------------------------|-----------|-----|--------------------|------------|
| (1) | (2) | (3) | (4) | (5) | (6) |
| 1. | <i>Alangium salvifolium</i> | Lf. | - | - | 15 mm/M |
| 2. | <i>Aristolochia indica</i> | Rt. | - | 22 50 mm/A mm/M | - |
| 3. | <i>Citrullus colocynthis</i> | Lf. | - | - | 20 mm/M |
| 4. | <i>Datura stramonium</i> | Lf. | - | 22 | - |

| | | | | | |
|----|--------------------------------|------|---|-----------------|-----------|
| 5. | <i>Ficus religiosa</i> | St.b | - | mm/M 20 | - |
| 6. | <i>Holoptelia integrifolia</i> | St.b | - | mm/M 10 | 25 |
| 7. | <i>Lawsonia innermis</i> | Lf. | - | mm/M 25 22 | mm/M - |
| 8. | <i>Ventilago madraspatana</i> | Rt.b | - | mm/A mm/M 20 | - |
| | | | | mm/M | |

A: Acetone pl. extract.

M: Methanol pl. extract.

A/M: Acetone and Methanol pl. extract.

In all, eight species only, out of 81 plant extract samples, exhibited a congealable inhibitory zone (20 mm) i.e. 10% of the total test samples.

Antifungal inhibition could be noticed in three species *Alangium salvifolium* is positive in both A/M, solvent extracts but distinct exhibition is at 15 mm diam. in methanol Presumably it must be antifungal and it may be preferred for further studies for conformation.

Antibacterial and antifungal inhibitory expression is observed in *Holoptelia integrifolia* St.b. extracts of both in M/A solvents respectively.

Although antibacterial expression showing 10 mm diam. inhibition only. Its antifungal expression is 25 mm diam. in methanol solvent similar to *Citrullus colosynthis*.

Regarding antibacterial inhibition, 6 species are positive with cognizable expression. Thus only 10% of the total samples are positive for antimicrobial immunity and correlating with the tribal medicinal uses specific for dermal (PC. 8), Arthritis and rheumatism, (PC. 5) and digestive disorders (PC. 12), etc.

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TRIBALS OF SILLHARIKOTA ISLAND



TRIBAL DOCTORS MATUVAYDIYAS SRIHARIKOTA



PLANT SPECIES (Expressed Above 20 mm Inhibition Zone)



Alangium salvifolium Wang.



Aristolochia indica L.



Citrullus colocynthis (L.) Schr.



Datura stramonium L.



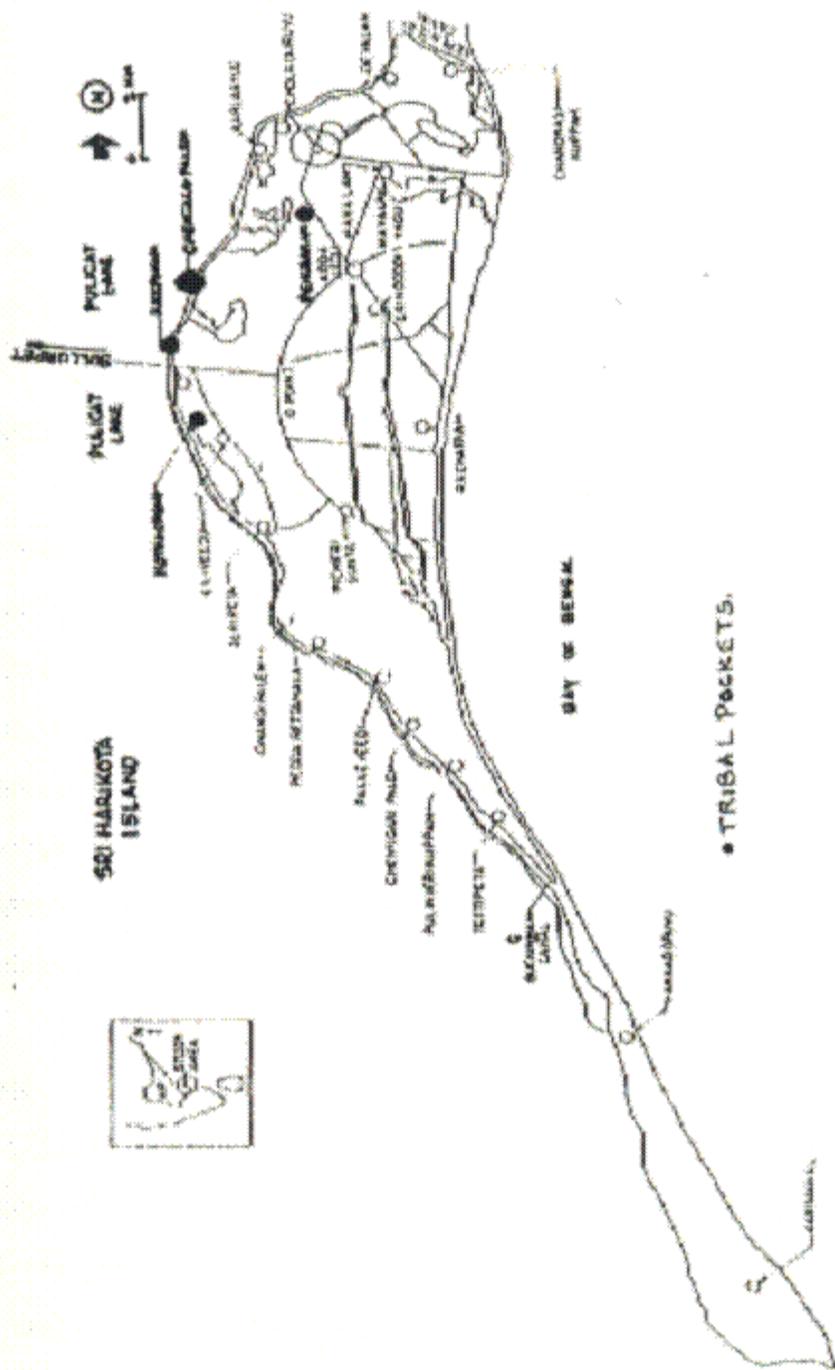
Holoptelia integrifolia (Roxb.) Planch.



Ficus religiosa L.



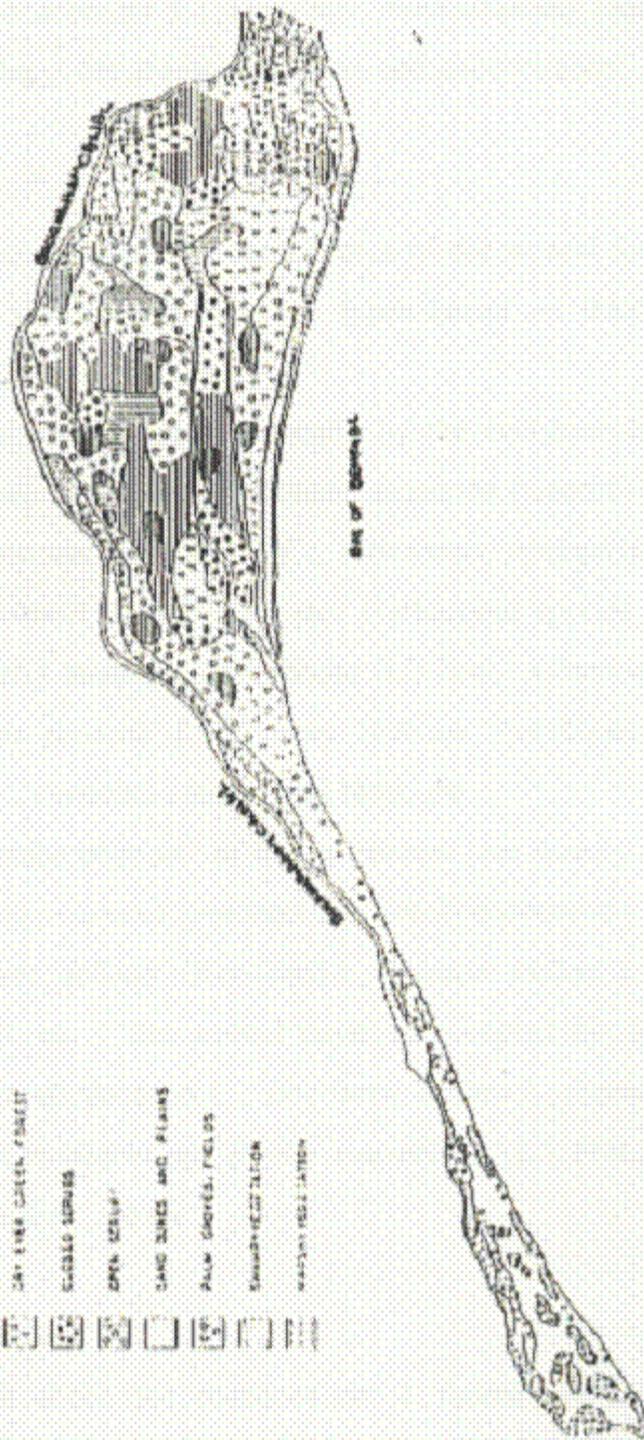
Lawsonia inermis L.



LOCALITY MAP

VEGETATION MAP OF THE SERENGETI ISLAND

-  CASUARINA PLANTATIONS
-  QUAILBUSH PLANTATIONS
-  CASHEW PLANTATIONS
-  DRY EVER GREEN FOREST
-  SCABLED SCRUBS
-  OPEN SCRUB
-  SAND BUNDS AND FLAKES
-  PALM GROVES, FIELDS
-  SHAMBHUKI PLANTATION
-  WASTED VEG. PATCHES



VEGETATION MAP - II

