MAURITIUS RESEARCH COUNCIL

VALIDATION OF THE ETHNOBOTANICAL INFORMATION OF SOME COMMONLY USED MEDICINAL PLANTS OF MAURITIUS

Final Report

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VALIDATION OF THE ETHNOBOTANICAL INFORMATION OF SOME COMMONLY USED MEDICINAL PLANTS OF MAURITIUS.

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TABLE OF CONTENTS.

List of Tables.

1.0 Introduction and Background.

The islands of Mauritius, Rodrigues and La Réunion from the Mascarene have Archipelago in the South West Indian Ocean, approximately 2000-km from the East Coast of Africa. The Republic of Mauritius comprises also the Island of Rodrigues while La Réunion is an Overseas French Department. Both Mauritius and Rodrigues are of volcanic origin and are totally surrounded by fringing coral reefs. The climate is sub-tropical with an average of 1500-mm rain annually. Rodrigues is the smallest and youngest of the three islands and is situated at around 574 km east of Mauritius.

The population of Mauritius of around 1.1 million inhabitants is basically of mixed origin with 69% Indo-Mauritians, 3% Sino-Mauritians, 28% general population, a category that includes Creoles of mixed African ancestry, Franco-Mauritians and members of various other European groups. Despite its small land area, Mauritius constitutes a distinctive botanical region. Of the 486 species which characterised the archipelago, 160 are endemic to Reunion, 150 to Mauritius and some 40 to Rodrigues and this would correspond to around 30% of the indigenous flora of each island (Cadet, 1980) and very few of these plants have been commercially utilised.

From 1990 to 1994, surveys have been carried out throughout Mauritius and Rodrigues in the context of a Project funded by the European Union, on the "Inventory and study of medicinal and aromatic plants of the States of the Indian Ocean". During this survey, several types of specialists concerned with healing using plants were interviewed. These were trained physicians, nurses, Indo-Mauritian herbalists, Chinese healers and doctors as well as lay people using medicinal plants.

The aim of this survey was to:

(a) Identify the general medical belief of the people.
(b) Collect information of remedies sold by professional herbalists and home remedies prepared by the lay population
(c) Assess the state of the distribution of the medicinal plants entering the traditional pharmacopoeia.

Five Mauritian healers along with 125 other informants throughout the island were interviewed. The informants provided more than 600 individual recipes. The informants and healers provided information on what plant/plant parts they used, preparation of the remedies, diseases treated, dose and regimen of the drugs. All of the specimens and samples were taken as they have been screened phytochemically as the data now form part of a database on the locally used medicinal plants. The herbarium specimens were identified by the Curator of the National Herbarium, Mr. J. Gueho and deposited at the Herbarium of Medicinal Plants at the Faculty of Science (Fakim, 1990; Gurib-Fakim *et al*, 1993-1997).

One issue in a survey of this kind is to what extent the healers can be trusted to give the correct information of the plants they used. If at least two informants independently reported the use of a plant in a remedy for a particular disease, the data was considered reliable. This work complements and updates the work of previous

authors (Daruty, 1886; Bouton, 1888; Baumer, 1979; Sussman, 1980; Adjanohoun *et al*, 1983).

The data obtained from the interview indicated that there is considerable knowledge and use of herbal remedies in Mauritius. Most of the people interviewed, had themselves taken herbal remedies and their parents and grandparents know how to prepare them. Unfortunately, the younger generation tend not to use medicinal plants but go for Western form of medicine. The survey also indicated that there are several types of part-time and full-time professional herbalists found on the island. These consist mainly of Indo-Mauritian herbalists who are full-time specialists and Mauritian stalls in major market places and Chinese pharmacists who are also full-time specialists and run stores in the major urban areas. Little information was collected from the latter as their information gets locked away into the "Chinese medicine" and they are generally more reticent about giving away data. It has also become apparent that all members of the Mauritian community utilise herbal medicine.

There are rules for properly preparing the various parts for use and for administering the remedies to assure a certain degree of success. The methods of preparation are simple. Most remedies are given orally as a decoction, tea or infusion usually made fresh for each dose. Poultices are the most common forms of external treatment. The plant parts, usually leaves, are prepared by passing them over a flame and rubbing the surface with cooking oil or paraffin before application to the skin. Other forms of external administration include baths, eyewashes and vaginal suppositories.

1.1 The World Health Approach.

During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggests that, in many developing countries just like Mauritius, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phyto-medicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs.

Even with this vast array of data, few medicinal plant species have been scientifically evaluated for their possible medical application. Safety and efficacy data are available for even a few plants, their extracts and their active ingredients, and the preparations containing them. Furthermore, in most countries the herbal medicine market is poorly regulated and herbal products are often neither registered nor controlled. Assurance of safety, quality and efficacy of medicinal plants and herbal products has now become a key issue in industrialised and in developing countries. Both the general consumer and health-care professionals need up-to-date information on the safety and efficacy of medicinal plants.

1.2 Objectives of this study:

Thus it is with this background that the project on the validation of some medicinal plants of Mauritius was carried out. The plants that have been selected are widely used and are important not just for Mauritius but for the region as well.

The purpose of this work is to provide:

- Preliminary scientific information on the safety and efficacy of the widely used medicinal plants in Mauritius. (The list of plants has been made from the survey carried out in Mauritius and Rodrigues between 1990-1994).

- The preliminary validation process was based on the following assays: anti-bacterial, anti-fungal screenings as well as the toxicity tests have been effected. The antibacterial assays were complemented with an assessment of their ability to contract and relax, *in vitro*, the ileum of rats and frogs.

- Provide a model and protocols for the preliminary evaluation of other medicinal plants.

- Study from the phytochemical and pharmacological point of view some of the endemic medicinal plants of Mauritius.

N.B. This work is the first of its kind in attempting to validate the medicinal plants of Mauritius. It will need to be updated with further detailed and more rigorous testing of the plants mentioned therein. The work reported here is purely of a scientific nature.

2.0 Selection of the herbal teas and medicinal plants.

The following herbal teas and medicinal plants have been selected mainly because of their popularity among the folk people using these plants against infectious diseases such as dysentery, diarrhoea, urinary tract infections, conjunctivitis etc.

Table 1. Recipes for the tisanes tested (Gurib-Fakim *et al*, 1995-1997).

Composition	Part used/Location where collection was made	
• ***Senna alata*** **tisane** *Senna alata* (katrepen) *Smilax anceps* (salsepareille)	 Leaves Leaves	 Le Morne Petrin
• ***Antidesma madagascariense*** **tisane** *Antidesma madagascariense*(Bois bigaignon) *Coix-lacryma jobi* (Collier cipaye) *Aploia theiformis* (Fandamane) *Erythroxylum laurifolium* (Bois de ronde) *Rhizophora mucronata* (Manglier) *Bidens pilosa* (Villebague) ***Piper*** **sp**. (Betel sauvage)	 Leaves Roots Leaves Stem bark Root Whole plant Leaves	 Le Petrin Phoenix Le Petrin Riviere Noire Le Morne Phoenix Le Petrin
• ***Vepris lanceolata*** **L. tisane** *Vepris lanceolata* (patte de poule)	Leaves and stem	Le Petrin
• ***Cassia fistula L.*** *Cassia fistula* (fleur cavadee)	Fruit pulp	Le Morne
• ***Ocimum tenuiflorum*** **tisane** *Ocimum tenuiflorum* (Tulsi) *Mentha-x-piperita* (Menthe) *Foeniculum vulgare* (Gros anis***)***	 Leaves Leaves Seeds	 Phoenix Phoenix
• ***Toddalia asiatic*****a tisane** *Toddalia asiatica* (patte poule piquant) *Zingiber officinalis* (Gingembre) *Cymbopogon citratus*	 Leaves Roots Leaves	 Le Petrin
• ***Solanum nigrum*** **L**. (Brede martin)	Leaves	Le Reduit
• ***Plectranthus amboinicus*** **L. (tisane)** *Plectranthus amboinicus* (Baume du Perou) *Cinnamomum camphora* (Camphrier)	 Leaves Leaves	 Le Petrin Le Petrin
• ***Cinnamomum camphora*** **Tisane** *Cinnamomum camphora* (Camphrier)	 Leaves	 De l'Eau Bouillie

Melia azedarach (Lilas de Perse) *Centella asiatica* (Boileau)	Leaves Whole plant	Moka Nouvelle Decouverte
• ***Tristemma mauritianum* Tisane** *Tristemma mauritianum* (Watook) *Centella asiatica* (Boileau) *Smilax anceps* (Salsepareille) *Senna occidentalis* (Cassepuante) *Catharanthus roseus* (Saponaire)	 Leaves Plant Leaves Plant Whole plant	 Le Petrin N. Decouverte Le Petrin Le Reduit Le Reduit

The other indigenous/endemic medicinal plants, which have also been screened, include:

	Plant names	**Family**
1.	*Acanthophoenix rubra*	Arecaceae
2.	*Agauria salicifolia*	Ericaceae
3.	*Cassine orientale*	Celastraceae
4.	*Ehretia petiolaris*	Celastraceae
5.	*Euphorbia pyrifolia*	Euphorbiaceae
6.	*Ficus reflexa*	Moraceae
7.	*Grangeria borbonica*	Chrysobalanaceae
8.	*Labourdonnaisia glauca*	Sapotaceae
9.	*Maytenus pyria*	Chrysobalanaceae
10.	*Olea lancea*	Oleaceae
11.	*Syzygium glomeratum*	Myrtaceae
12.	*Tarenna borbonica*	Rubiaceae
13.	*Turraea casimiriana*	Meliaceae

2.1 Collection of plant materials.

Most of the exotic plants have been collected from the wild while the indigenous plants were obtained from the Nature Reserves as mentioned earlier on. These plants were cleaned free from mosses, insects, soil and other particles. Voucher specimens of each collected plant was sent to the National Herbarium at the Mauritius Sugar Industry and Research Institute (MSIRI) for identification purposes. The identity of the plants were confirmed either by the Curator of the National Herbarium–Mr J. Gueho and/or Ms D. Florens.

The plants were then separated into different parts such as leaves and stems and placed in a drying cabinet at 45° C for 3-5 days until complete dryness. The dried leaf and stem plant materials were then ground separately using an electric grinder. The mass of the yielded plant material was determined and the plant material was kept in clearly labeled airtight plastic containers.

2.2 Preparation of plant extracts

2.2.1 Tisanes.

Tisanes or herbal teas were prepared by simple decoction based on the recipes given for tisanes (Gurib-Fakim *et al*, 1995-1997). After decoction, the water was removed *in vacuo* and the extracts were kept in sample bottles for further use.

2.22 Leaf and stem fractions

The leaf and stem fractions were obtained by simple decoction whereby equal mass of the leaf and stem parts was allowed to boil in water. The resulting filtrate was concentrated *in vacuo* and the mass yielded was weighed.

The different stem and leaf fractions were prepared as follows:

The powdered plant material (25-40g) was placed in a Soxhlet collector and extracted using a gradient elution of the following solvents (200 ml each): Hexane, Chloroform, Chloroform:Methanol and Methanol as solvents respectively. The separated yielded solutions were evaporated *in vacuo* to give the concentrated fractions. The yield was calculated as g extract / g dry plant material.
All the prepared extracts were kept at 4°C for further use.

2.3 Phytochemical screenings (Gurib-Fakim *et al*, 1997).

A series of chemical tests were performed on the extracts to detect any presence of alkaloids, phenols, tannins, terpenes, saponins, anthraquinones, coumarins, flavones and related flavonoid glycosides as well as cyanogenic glycosides and leucoanthocyanins.

2.3.A Screening for alkaloids.

The plant extracts were spotted on TLC plates and allowed to elute using the following eluants: ether/methanol/acetone/ammonia solution in the ratio of 9:2:8:1. After drying, the TLC plates were sprayed with Dragendorff's reagent. The presence of pink, orange, pinkish orange or pinkish brown spots on a yellow background was taken to be positive for the presence of alkaloids.
Turbidity or precipitation tests with Dragendorff's reagent were also performed on the plant extracts. Turbidity and precipitation test was used to confirm presence of alkaloids.

2.3B Screening for anthraquinones.

1 gram of the concentrated plant extract was dissolved in distilled water (30 ml). The solution was then filtered and the filtrate was extracted with benzene (10 ml). 5 ml of ammonium hydroxide was added to the benzene extract in a test tube and the mixture was shaken. A red coloration in the lower aqueous layer indicated the presence of anthraquinones.

2.3C Screening for phenols.

The concentrated plant extract was spotted on a TLC plate and was allowed to elute with the following eluant: chloroform/methanol (4:1). After drying, the TLC plate was sprayed with a solution of vanillin (1 g of vanillin in 10 ml of concentrated hydrochloric acid). Appearance of pink spots on the plate after spraying was taken as evidence for presence of phenols.

2.3D Screening for terpenes.

TLC was performed on the concentrated plant extract using ethyl acetate/hexane mixture as eluant. The proportion of ethyl acetate and hexane was varied so as to obtain the best chromatogram. After elution, the TLC plate was dried and sprayed with freshly prepared Liebermann-Burchard Reagent. The plate was heated in an oven (60-80° C) for about 5-20 minutes. Appearance of a blue of violet or pink colour was indicative of the presence of terpenes.

2.3E Screening for cyanogenic glycosides.

2.5 g of dried powdered plant material was moistened with distilled water in a 125-ml conical flask followed by 1 ml of chloroform. A strip of filter paper was dipped in freshly prepared sodium picrate solution (sodium carbonate: 5 g and piric acid: 0.5 g in 100 ml of distilled water). It was imediately placed at the mouth of the conical flask just above the plant material. Care was taken to prevent the filter paper from touching the sides of the flask and the plant material. The conical flask was closed and allowed to stand at 35° C for 3 hours. The filter paper changed from yellow to red if cyanogenic glycosides were present in the plant due to evolution of hydrogen cyanide gas. If the filter paper remained yellow after 3 hours, absence of cyanogenic glycosides was concluded.

2.3F Screening for saponins.

For testing for the presence of saponins, 0.5 g of the dried powdered plant material was treated with 10 ml of distilled water for 5 minutes at 100° C in a test tube. It was then cooled and shaken vigorously. Formation of froth (1-2 cm) persisting for at least 30 minutes was taken as a positive test for saponins.

S2.3 G Screening for tannins.

The prepared plant extract was filtered. To the filtrate in a test tube, a mixture of ferric chloride (3%) and potassium ferricyanide (3%), (1:1 solution) was added. The appearance of a blue-black, green or bluish-green coloration was taken to be positive for the presence of tannins.

2.3H Screening for coumarins.

The plant extract was made alkaline by addition of ammonium hydroxide and then observed under ultra-violet light. A blue, green or violet fluorescence of the basified extract was taken as a positive test for coumarins.

2.3I Screening for flavones and related flavonoid glycosides.

15 ml of petroleum ether was added to the concentrated plant extract to remove pigments. The solution was then filtered. The procedure was repeated until all the pigments were removed. The residue was then dissolved in 30 ml of ethanol (95%) and the resulting solution filtered. To 3 ml of the filtrate in a test tube, concentrated hydrochloric acid 0.5 ml) was added followed by 3-4 magnesium turnings. The appearance of a red or orange coloration after 10 minutes was taken as a positive test for flavones and related flavonoid glycosides.

2.3J Screening for leucoanthocyanins.

The procedure was the same as for flavones and related flavonoid glycosides except that after the addition of concentrated hydrochloric acid, the test tube was allowed to stand in a boiling water bath for 30 minutes. After this period of time, the test tube was allowed to cool and the colour of the solution was noted. A red coloration indicated the presence of leucoanthocyanins.

2.4 Antimicrobial screening

Antimicrobial activity was determined by the Dilution method (Mitscher *et al*., 1972). All the described procedures that require sterility were carried out in accordance with the guidelines for aseptic work, implying use of laminar air flow (LAF) and pre-sterilised glasswares.

2.5 Test organisms

The following microbiological agents were used as test organisms:

Bacteria	**Fungi**
Escherichia coli	*Candida albicans*
Pseudomonas aeruginosa	*Aspergillus niger*
Salmonella typhimerium	
Staphylococcus aureus	

Cultures of the 4 bacteria and one fungus *C.albicans* were maintained respectively in cooked ox meat broth solution and Sabouraud dextrose agar slant at 5° C. *A. niger* was kept on a Sabouraud dextrose agar slant at 27° C. Prior to testing, new subcultures of bacteria and fungi had to be ensured.

2.6 Antibacterial assay.

The assay was performed using agar plates prepared by mixing solutions of plant extract with liquefied Muller-Hinton agar. To limit the risk of random errors, duplicate determinations were carried out on every extract along with a negative control and a positive control (Pedersen, 1999).
0.32 g of the plant extract was dissolved in 1 ml of solvent depending on which fraction was being used as shown below:

Plant fraction	Solvent used to dissolve the plant extract
hexane	0.5 ml chloroform+0.25 ml Tween 80+0.25 ml water
Chloroform: methanol	1 ml methanol+1 ml water
Methanol	1 ml methanol+1 ml water

The test-tube was vigorously stirred by means of a Vortex mixer. The prepared sample was then mixed with 9.0 ml of melted Muller-Hinton agar in sterilised disposable 10.0-ml petri-dish. The petri-dish was well swirled to ensure complete mixing. In addition two control plates containing 9.0-ml Muller-Hinton agar and 1.0 ml of the respective solvent were prepared. After solidification, one drop of each of the bacteria suspensions was inoculated on the plates with a platinum loop. The drops were allowed some fifteen minutes to get fully absorbed in the agar and the plates were incubated overnight. The results were taken after 24 hours.
Following use of the above procedure, a concentration of 32 mg/ml of the plant extract in the agar was obtained.

2.7 Positive control

Ciproglen (Glenmark Phamaceuticals Ltd., India) and Amoxyl (SmithKline Beecham Pharmaceuticals, Brentford England) tablets were used as positive controls.

2.8 Anti-fungal assay

Except for the different growth medium used, the procedures were the same as described for the bacterial assay. Plates were prepared using Sabouraud dextrose agar as solid medium for the fungi. After inoculation with the two fungi, the plates were incubated for a period of 48 hours.

2.9 Positive control

Nystatin (Bristol-Myers Squibb) and Nizoral (Janssen, U.K.) tablets were used as positive controls.

3.0 Determination of Minimum Inhibitory Concentration

Extracts found to be active against the bacteria and fungi at an initial concentration of 32 mg/ml were further investigated to determine the appropriate minimum inhibition concentration (MIC). This was done by using a series of dilutions to give agar plates containing plant extracts in the concentrations of 16 mg/ml, 8 mg/ml, 4 mg/ml, 2 mg/ml, 1 mg/ml and 0.5 mg/ml respectively. The MIC value was taken as the weakest concentration in the descending concentration series showing no apparent growth (Jelager, 1996).

3.1 Screening on tannin-free extracts

Extracts showing anti-microbial properties were investigated as to whether the activity could be ascribed to tannins that are known to possess anti-microbial properties. So the plant extracts were further treated to remove tannins present as described below and the tannin-free extracts were again tested for anti-microbial activities (Jelager *et al*, 1998).

3.1A Methodology

The amount of the plant extracts to be tested was dissolved in 25 ml of warm distilled water. The insoluble fractions were removed by filtration. 2 % aqueous gelatine solution was added dropwise to the filtrate until no more precipitation was apparent. The filtrate was then centrifuged for 20 minutes with short intervals so that it was possible to judge whether a drop of gelatine would cause further precipitation or not. The supernatant was then decanted and water was removed *in vacuo*.

3.2 Screening for pharmacological properties.

Adult male Sprague-Dawley rats and weighing between 50-100 g were killed by a severe blow to the head. Strips from aorta and small intestine were prepared and mounted in an organ bath containing 25 ml of Krebs-Henseleit buffer as described by Subratty *et al*., (1999). The composition (mM) of the buffer was as follows: NaCl 118; KCl 4.7; $CaCl_2.H_2O$ 2.5; $MgSO_4.7H_2O$ 1.2; KH_2PO_4 25; $NaHCO_3$ 25; Na_2EDTA 9.7 mg/l and glucose (2 g/l). To prevent blood clot formation in the dissected organ strips, 2 ml of Heparin (5,000 IU/L) were added to the buffer in a petridish. The tissue bath solution was maintained at 37° C in a thermostat water bath. A gas mixture of 95 % O_2 and 5 % CO_2 was continuously bubbled in the buffer and the pH was adjusted to 7.45. Two stainless steel hooks were inserted into the aorta lumen, one was fixed while the other was connected to a transducer. Isometric contractile responses were recorded via a force transducer connected to a multipen recorder (Rikadenki MODEL R50; Japan). Organ strips were allowed to equilibrate in the medium for 20 minutes and maintained under an optimal tension of 1.5 g.

3.2A Preparing plant extract for assay

0.132 g of the plant extract obtained by Soxhlet extraction was dissolved in 1 ml of the respective solvent as used in the microbiological assay in section 2.5. The sample was well stirred by means of a vortex mixer.

3.3 Challenging the strips

After equilibration of the strip in the in the organ bath buffer, 100 µl of the prepared plant extract was added to the organ bath followed by another 100 µl at three minutes interval until all the prepared plant extract (1 ml) was used.

3.4 Control experiment

For each series of experiments, a parallel control strip was included and challenged with the respective solvent used for extraction at three minutes interval.

3.5 Toxicity

The ten tisanes were investigated for their toxicity levels in twenty rats. All tests were carried in duplicate. For each two tisanes, two rats injected with distilled water were used as controls.

3.51 Methodology

Depending on the weight of the experimental animals, a stock solution of each tisane was prepared accordingly. The weight of the experimental animals and control were taken as baseline. Using sterilised disposable syringes, 1 ml of the tisane was injected sub-cutaneously in the rats. This represented an initial dose of 30 µg/kg.
1 ml of distilled water was then injected in the control. From the first day of injection, the weights of the organisms were taken daily for a week. Following a week, the organisms were injected with a second dose of tisane of concentration of 60 µg/kg. The controls were injected with the same amount of distilled water. The weights of the animals were taken daily. Subsequently on the third week the dose of tisane used was 90 µg/kg and a final dose of 120 µg/kg was used in the fourth week.

Preliminary results from the screens of indigenous/endemic medicinal plants.

Table 2: Results from the initial antimicrobial screening.
(NG = No growth; G = Growth, RG = Reduced growth)

Plant species	Plant part	*E.coli*	*P.aeruginosa*	*S.typhimerium*	*S.aureus*	*A.niger*	*C.albicans*
Acanthophoenix rubra	Leaves	G	G	G	NG	G	G
Agauria salicifolia	Leaves	G	NG	G	NG	G	G
	Stem	G	RG	G	NG	NG	G
Cassine orientale	Young leaves	NG	NG	NG	NG	RG	G
	Mature leaves	G	G	G	NG	G	G
	Stems	NG	NG	NG	NG	G	G
Ehretia petiolaris	Leaves	G	NG	G	G	G	G
	Stem	G	RG	G	G	RG	G
	Fruits	G	NG	G	G	RG	G
Euphorbia pyrifolia	Leaves	G	G	G	G	G	G
	Stems	G	RG	G	NG	G	G
Ficus reflexa	Leaves	G	NG	RG	NG	RG	G
	Stem	G	G	G	G	G	G
Grangeria borbonica	Leaves	G	NG	NG	NG	NG	G
	Stems	G	NG	NG	NG	G	G
Labourdonnaisia glauca	Leaves	RG	NG	NG	NG	NG	G
	Stems	NG	NG	G	NG	G	G
Maytenus pyria	Leaves	G	G	G	NG	G	G
	Stems	G	NG	NG	NG	NG	G
Olea lancea	Leaves	G	G	G	NG	G	G
	Stems	G	NG	NG	NG	NG	G
Syzygium glomeratum	Leaves	NG	NG	NG	NG	NG	G
	Stems	G	NG	G	NG	NG	G
Tarenna borbonica	Young leaves	G	RG	G	NG	G	G
	Mature leaves	G	G	G	G	RG	G
	Stems	G	RG	G	G	G	G
Turraea casimiriana	Young leaves	G	G	G	G	G	G
	Mature leaves	G	RG	G	NG	NG	G
	Stems	RG	NG	NG	NG	G	G

Table 3: MIC–determination

Plant species	Plant part	*E.coli*	*P.aeruginosa*	*S.typhimerium*	*S.aureus*	*A.niger*	*C.albicans*
Acanthophoenix rubra	Leaves	-	-	-	1 mg/ml	-	-
Agauria salicifolia	Leaves	-	8mg/ml	-	8 mg/ml	-	-
	Stem	-	Nd	-	1 mg/ml	-	4mg/ml
Cassine orientale	Young leaves	Nd	4 mg/ml	8 mg/ml	1 mg/ml	-	Nd
	Mature leaves	-	1 mg/ml	-	-	-	-
	Stems	2 mg/ml	1 mg/ml	8 mg/ml	1 mg/ml	-	-
Ehretia petiolaris	Leaves	-	8 mg/ml	-	-	-	-
	Stem	-	Nd	-	-	-	Nd
	Fruits	-	8 mg/ml	-	-	-	Nd
Euphorbia pyrifolia	Leaves		-	-	-	-	-
	Stems	-	Nd	-	0,5 mg/ml	-	-
Ficus reflexa	Leaves	-	-	4 mg/ml	2 mg/ml	-	Nd
	Stem	-	-	-	-	-	-
Grangeria borbonica	Leaves	-	2 mg/ml	8 mg/ml	2 mg/ml	-	8 mg/ml
	Stems	-	1 mg/ml	8 mg/ml	1 mg/ml	-	-
Labourdonnaisia glauca	Leaves	-	8 mg/ml	8 mg/ml	1 mg/ml	-	-
	Stems	8 mg/ml	4 mg/ml	-	8 mg/ml	-	-
Maytenus pyria	Leaves	-	-	-	2 mg/ml	-	-
	Stems	-	2 mg/ml	4 mg/ml	1 mg/ml	-	8 mg/ml
Olea lancea	Leaves	Nd	2 mg/ml	8 mg/ml	2 mg/ml	-	8 mg/ml
	Stems	-	4 mg/ml	-	4 mg/ml	-	4 mg/ml
Syzygium glomeratum	Leaves	8 mg/ml	1 mg/ml	4 mg/ml	1 mg/ml	8 mg/ml	-
	Stems	-	2 mg/ml	-	2 mg/ml	-	8 mg/ml
Tarenna borbonica	Young leaves	-	Nd	-	8 mg/ml	-	-
	Mature leaves	-	-	-	-	-	-
	Stems	-	Nd	-	-	-	-

Turraea casimiriana	Young leaves	-	Nd	-	-	-	-
	Mature leaves	-	Nd	-	4 mg/ml	-	8 mg/ml
	Stems	Nd	1mg/ml	8 mg/ml	2 mg/ml	-	-

(-) = No activity; Nd = Not determined; Xmg/ml = Activity observation at reported concentration.

Table 4: Results from the tannin precipitation.

Plant species	**Plant part**	**Tannins**	**Tannin precipitation**	**Activity against**	**After tannin pption: Activity against**
Acanthophoenix rubra	Leaves	+	+	*S. aureus*	*S. aureus*
Agauria salicifolia	Leaves	+	+	*P. aeruginosa, S. aureus*	- -
	Stems	+	+	“ “	- -
Cassine orientale	Y. leaves	+	+	*E.coli, P. aeruginosa, S. typhumurium, S. aureus*	-, *P. aeruginosa*, -, *S. aureus*
	M. leaves	Trace	+	*S. aureus*	*S. aureus*
	Stems	+	+	*E. coli, P. aeruginosa, S. typhumurium, S. aureus*	*E. coli, P. aeruginosa*, -, *S. aureus*
Ehretia petiolaris	Leaves	+	-	*P. aeruginosa*	-
	Stems	+	-	*P. aeruginosa*	*P. aeruginosa*
	Fruits	Trace	-	*P. aeruginosa*	-
Euphorbia pyrifolia	Leaves	+	Nd	-	-
	Stems	-	Traces	*P. aeruginosa, S. aureus*	-, -
Ficus reflexa	Leaves	-	+	*P. aeruginosa, S. typhumurium, S. aureus*	-, -, -
	Stems	-	Nd	-	-
Grangeria borbonica	Leaves	+	+	*E. coli, P. aeruginosa, S. typhumurium, S. aureus*	*P. aeruginosa*
	Stems	+	+	*E. coli, P. aeruginosa, S. typhumurium, S. aureus*	*P. aeruginosa*, -, -, -

Labourdonnaisia glauca	Leaves	+	+	*E. coli, P. aeruginosa, S. typhumurium, S. aureus*	*-, P. aeruginosa, S. typhumurium, S. aureus*
	Stems	+	+	*S. aureus,*	-
Maytenus pyria	Leaves	+	+	*S. aureus*	-
	Stems	+	+	*P. aeruginosa, S. typhumurium, S. aureus,*	*P. aeruginosa, -, S. aureus*
Olea lancea	Leaves	+	+	*E. coli, P. aeruginosa, S. typhumurium, S. aureus*	*-, P. aeruginosa, -, S. aureus*
	Stems	+	-	*E. coli, P. aeruginosa, S. typhumurium, S. aureus*	*-, P. aeruginosa, -, S. aureus.*
Syzygium glomeratum	Leaves	+	+	*E. coli, P.aeruginosa, S. typhumurium, S. aureus*	*-, -, -, S. aureus*
	Stems	+	+	*P. aeruginosa, S. aureus*	*P. aeruginosa, -*
Tarenna borbonica	Y. leaves	+	+	*P. aeruginosa, S. aureus*	-
	M. leaves	-	Nd	-	-
	Stems	+	Trace	*P. aeruginosa*	-
Turraea casimiriana	Y. leaves	-	Nd	-	-
	M. leaves	+	+	*P. aeruginosa, S. aureus*	*P. aeruginosa*
	Stems	+	+	*E. coli, P. aeruginosa, S. typhumurium, S. aureus*	*-, P. aeruginosa, -, S. aureus*

(+) = Extract has been found to be active
(-) = Extract has been found to be inactive
Nd = Test Not done

Table 5: Results from the phytochemical screenings of indigenous plants.

Plant names	Plant parts	Phytochemicals found
Acanthophoenix rubra	Leaves	Tannins, traces of saponins, flavonoids, Proanthocyanidins, iridoids and traces of triterpenes.
Agauria salicifolia	Leaves	Traces of flavonoids, proanthocyanidins, tannins, iridoids and triterpenes
	Stems	Proanthocyanidins, traces of saponins, flavonoids, tannins, iridoids, triterpenes
Cassine orientale	Young Leaves	Traces of tannins, proanthocyanidins, flavonoids, iridoids, saponins
	Mature leaves	Traces of tannins, proanthocyanidins, flavonoids, iridoids
	Stems	Tannins, proanthocyanidins, flavonoids, traces of iridoids and saponins
Ehretia petiolaris	Leaves	Tannins, traces of saponins and iridoids
	Stems	Tannins and traces of saponins, flavonoids and iridoids
Euphorbia pyrifolia	Leaves	Tannins, traces of flavonoids and iridoids
	Stems	Traces of flavonoids and proanthocyanidins
Ficus reflexa	Leaves	Flavonoids and proanthocyanidins
	Stems	Proanthocyanidins and saponins
Grangeria borbonica	Leaves	Tannins, iridoids, flavonoids, proanthocyanidins
	Stems	Proanthocyanidins, flavonoids, iridoids, tannins and traces of saponins
Labourdonnaisia glauca	Leaves	Tannins, proanthocyanidins, iridoids, flavonoids, triterpenes
	Stems	Tannins, proanthocyanidins, flavonoids, triterpenes and traces of saponins
Maytenus pyria	Leaves	Flavonoids, saponins, proanthocyanidins, iridoids and tannins
	Stems	Flavonoids, saponins, proanthocyanidins, iridoids and tannins

Olea lancea	Leaves	Saponins, tannins and traces of proanthocyanidins
	Stems	Saponins, tannins and traces of flavonoids and iridoids
Syzygium glomeratum	Leaves	Tannins, proanthocyanidins, iridoids and traces of saponins
	Stems	Tannins, flavonoids, proanthocyanidins and iridoids
Tarenna borbonica	Young Leaves	Flavonoids, proanthocyanidins, tannins, iridoids, and traces of triterpenes and saponins
	Mature leaves	Iridoids and traces of saponins
	Stems	Flavonoids, proanthocyanidins, tannins, iridoids and traces of saponins
Turraea casimiriana	Young Leaves	Traces of iridoids
	Mature leaves	Tannins and traces of iridoids, flavonoids, and proanthocyanidins
	Stems	Flavonoids, proanthocyanidins, saponins, tannins and iridoids

Further to these preliminary screenings of the indigenous/endemic medicinal plants, the fractions obtained from the plants listed below have been analysed further and are:

1. *Antidesma madagascariensis*
2. *Faujasiopsis flexuosa*
3. *Toddalia asiatica*
4. *Vepris lanceolata*
5. *Antidesma madagascariensis*

Table 6: Antimicrobial activities of *Antidesma madagasc*ariense extracts. (Test concentration: 32 mg/ml)

Plant Extracts (32 mg/ml)	***E.coli***	***P.aeruginosa***	***S.typhimerium***	***S.aureus***	***A.niger***	***C.albicans***
Leaf and stem (crude water extract)	NG	NG	NG	NG	0.4 – 0.3 0.2 – 0.2	0.3 – 0.3 0.4 – 0.5
Stem Fractions						
Hexane fraction	0.3 – 0.3	NG	0.3 – 0.4 0.5 – 0.4	NG	0.7 – 0.6 0.2 – 0.4	0.5 – 0.6 0.8 – 0.9
Chloroform:methanol fraction(1:1)	0.4 – 0.3 0.4 – 0.5	NG	0.3 – 0.2 0.2 – 0.2	NG	NG	0.2 – 0.3 0.4 – 0.5
Methanol fraction	NG	NG	NG	NG	0.4 – 0.3 0.5 – 0.4	0.5 – 0.6 0.4 – 0.6
Leaf fractions						
Hexane fraction	0.6 – 0.7 0.6 – 0.5	0.8 – 0.9 0.4 – 0.5	0.4 – 0.5 0.4 – 0.4	0.3 – 0.3 0.3 – 0.5	0.5 – 0.6 0.7 – 0.7	1.0 – 0.9 0.7 – 0.8
chloroform:methanol fraction(1:1)	0.3 – 0.4 0.6 – 0.7	NG	0.2 – 0.3 0.2 – 0.2	NG	NG	1.0 – 1.1 0.5 – 0.6
Methanol fraction	NG	NG	NG	NG	NG	0.1 – 0.2 0.2 – 0.3

Table 7: Antimicrobial activities of *Faujasiopsis flexuosa* extracts (Test concentration: 32 mg/ml)

Plant extracts (32 mg/ml)	***E.coli***	***P.aeruginosa***	***S.typhimerium***	***S.aureus***	***A.niger***	***C.albicans***
Leaf and stem (crude water extract)	NG	NG	NG	NG	0.5 – 0.5 0.5 – 0.6	0.4 – 0.3 0.5 – 0.6
Stem fractions						
Hexane fraction	1.0 – 1.1 0.7 – 0.9	0.5 – 0.5 0.7 – 0.8	0.2 – 0.4 0.8 – 1.0	NG	0.3 – 0.5 0.2 – 0.3	0.6 – 0.8 0.7 – 0.7
chloroform:methanol (1:1) fraction	NG	NG	NG	NG	NG	0.8 – 0.6 0.5 – 0.6
Methanol fraction	0.6 – 0.6 0.7 – 0.6	1.0 – 1.1 0.5 – 0.4	1.0 – 0.9 1.0 – 0.7	NG	0.4 – 0.5 0.2 – 0.3	0.2 – 0.2 0.4 – 0.5
Leaf Fractions						
Hexane fraction	0.3 – 0.3 0.1 – 0.2	0.5 – 0.6 0.4 – 0.5	0.6 – 0.7 0.4 – 0.5	0.5 – 0.5 1.0 – 0.8	NG	0.3 – 0.3 1.0 - 0.8
chloroform:methanol (1:1)fraction	NG	NG	NG	NG	NG	0.9 – 0.6 0.7 – 0.5
Methanol fraction	NG	NG	NG	NG	NG	0.3 – 0.2 0.2 – 0.2

Table 8: Antimicrobial activities of *Toddalia Asiatica* extracts (Test concentration: 32 mg/ml)

Plant extracts (32 mg/ml)	***E.coli***	***P.aeruginosa***	***S.typhimerium***	***S.aureus***	***A.niger***	***C.albicans***
Leaf and stem (crude water extract)	0.3 – 0.2 0.2 – 0.2	NG	0.2 – 0.3 0.3 – 0.4	NG	0.7 – 0.7 0.8 – 0.9	0.6 – 0.8 0.7 – 0.9
Stem fractions						
Hexane fraction	0.7 – 0.6 0.4 – 0.4	0.4 – 0.6 0.7 – 0.5	0.8 – 0.6 0.2 – 0.3	0.3 – 0.4 0.3 – 0.2	0.2 – 0.4 0.7 – 0.5	0.9 – 1.0 0.8 – 0.8
chloroform:methanol fraction(1:1)	1.0 – 0.9 1.0 – 0.7	NG	0.5 – 0.5 0.5 – 0.6	NG	NG	0.2 – 0.2 0.2 – 0.2
Methanol fraction	1.0 – 0.9 0.9 – 0.8	0.6 – 0.7 0.8 – 0.6	0.6 – 0.6 0.5 – 0.4	0.2 – 0.3 0.3 – 0.3	0.1 – 0.2 0.3 – 0.2	1.0 – 0.9 0.4 – 0.6
Leaf fractions						
Hexane fraction	0.4 – 0.3 0.7 – 0.5	NG	0.4 – 0.2 0.7 – 0.9	NG	NG	NG
chloroform:methanol fraction(1:1)	NG	NG	NG	NG	NG	-
Methanol fraction	1.1 – 1.0 0.8 – 0.8	NG	0.7 – 0.9 0.8 – 1.2	NG	NG	0.6 – 0.5 0.6 – 0.4

Table 9: Antimicrobial activities of *Vepris lanceolata* extracts (Test concentration: 32 mg/ml)

Plant extracts 32mg/ml	***E.coli***	***P.aeruginosa***	***S.typhimerium***	***S.aureus***	***A.niger***	***C.albicans***
Leaf and stem (crude water extract)	0.9 – 1.0 0.7 – 0.9	0.6 – 0.5 0.4 – 0.4	0.4 – 0.3 0.5 – 0.8	0.4 – 0.5 0.8 – 0.7	0.7 – 0.6 1.0 – 0.6	0.6 – 0.7 1.1 – 0.8
Stem fractions						
Hexane fraction	0.2 – 0.3 0.3 – 0.4	NG	0.3 – 0.3 0.4 – 0.3	NG	0.4 – 0.5 0.6 – 0.7	0.7 – 0.8 0.5 – 0.6
Chloroform:methanol fraction(1:1)	0.7 – 0.8 0.7 – 0.6	NG	1.0 – 0.9 0.4 – 0.3	NG	NG	NG
Methanol fraction	0.5 – 0.7 0.6 – 0.6	0.4 – 0.3 0.6 – 0.6	0.4 – 0.5 0.7 – 0.8	0.9 – 1.0 0.5 – 0.5	NG	NG
Leaf fractions						
Hexane fraction	1.0 – 0.9 1.0 – 0.6	0.5 – 0.5 0.7 – 0.9	0.7 – 0.7 0.4 – 0.3	0.3 – 0.4 0.6 – 0.4	0.8 – 0.9 0.5 – 0.4	1.0 – 0.9 1.0 – 0.8
Chloroform:methanol fraction(1:1)	1.0 – 0.8 0.7 – 0.6	0.4 – 0.3 0.3 – 0.3	0.4 – 0.5 0.4 – 0.4	NG	NG	0.7 – 0.6 0.5 – 0.5
Methanol fraction	0.4 – 0.4 0.5 – 0.7	0.7 – 0.5 0.4 – 0.2	0.3 – 0.4 0.7 – 0.9	0.6 – 0.7 0.8 – 1.0	NG	0.4 - 0.6 0.5 – 0.5

Table 10: Antimicrobial activities of tannin-free extracts of *Antidesma Madagascariense* (Test concentration: 32 mg/ml)

Plant extracts (32 mg/ml)	***E.coli***	***P.aeruginosa***	***S.typhimerium***	***S.aureus***	***A.niger***	***C.albicans***
Leaf and stem (crude water extract)	1.1 – 1.0 0.6 – 0.9	0.4 – 0.5 0.8 – 0.9	NG	NG		
Stem fractions						
Hexane fraction		0.3 – 0.2 0.4 – 0.6		0.7 – 1.1 0.5 – 0.6	0.2 – 0.3 0.2 – 0.4	
Chloroform:methanol fraction(1:1)	0.3 – 0.2 0.3 – 0.3	NG	0.8 – 0.9 0.4 – 0.2	NG	NG	
Methanol fraction	NG	NG	NG	NG	0.6 – 0.8 0.2 – 0.4	
Leaf fractions						
Hexane fraction						
chloroform:methanol fraction(1:1)		0.5 – 0.5 1.2 – 1.1	0.4 – 0.5 0.4 – 0.3	0.7 – 0.9 0.8 – 0.9	0.3 – 0.3 0.2 – 0.5	
Methanol fraction	0.2 – 0.2 0.4 – 0.6	0.7 – 0.7 0.5 – 0.6	NG	NG	NG	

Table 11: Antimicrobial activities of tannin-free extracts of *Faujasiopsis flexuosa* (Test concentration: 32 mg/ml)

Plant Extracts (32 mg/ml)	***E.coli***	***P.aeruginosa***	***S.typhimerium***	***S.aureus***	***A.niger***	***C.albicans***
Leaf and stem (crude water extract**)**	0.5 – 0.6	0.8 – 0.9 0.3 – 0.2	0.7 – 0.7 0.7 – 0.6	NG		
Stem fractions						
Hexane fraction				0.2 – 0.2 0.2 – 0.5		
Chloroform:methanol fraction(1:1)	NG	NG	NG	NG	0.4 – 0.4 0.4 – 0.7	
Methanol fraction				0.2 – 0.5 0.4 – 0.8		
Leaf fractions						
Hexane fraction					0.9 – 1.1 0.6 – 0.6	
chloroform:methanol fraction(1:1)	NG	NG	NG	NG	NG	
Methanol fraction	1.2 – 1.3 0.7 – 1.0	0.4 – 0.4 0.3 – 0.4	NG	0.5 – 0.6 0.2 – 0.4	NG	

NG: no growth

Table 12: Antimicrobial activities of tannin-free extracts of *Toddalia asiatica* (Test concentration: 32 mg/ml)

Plant Extracts (32 mg/ml)	***E.coli***	***P.aeruginosa***	***S.typhimerium***	***S.aureus***	***A.niger***	***C.albicans***
Leaf and stem (crude water extract**)**	0.7 – 0.6 0.5 – 0.4	0.4 – 0.7 0.4 – 0.4	0.2 – 0.2 0.4 – 0.3	NG		
Stem fractions						
Hexane fraction				0.5 – 0.8 0.7 – 0.3		
Chloroform:methanol fraction(1:1)	NG	NG	NG	NG	0.4 – 0.3 0.6 – 0.7	
Methanol fraction				0.2 – 0.3 0.2 – 0.2		
Leaf fractions						
Hexane fraction					1.2 – 0.5	
chloroform:methanol fraction(1:1)	NG	NG	NG	NG	NG	
Methanol fraction	0.9 – 0.8 0.8 – 0.8	0.4 – 0.6 0.7 – 0.8	NG	0.6 – 0.5 0.5 – 0.2	NG	

NG: no growth

Table 13: Antimicrobial activities of tannin-free extracts of *Vepris lanceolata* (Test concentration: 32 mg/ml)

Plant extracts (32 mg/ml)	***E.coli***	***P.aeruginosa***	***S.typhimerium***	***S.aureus***	***A.niger***	***C.albicans***
Leaf and stem (crude water extract)						
Stem fractions						
Hexane fraction		0.2 – 0.4 0.7 – 0.8		1.0 – 1.1 0.7 – 0.7		
Chloroform:methanol fraction(1:1)		0.5 – 0.6 0.5 – 0.5		0.3 – 0.4 0.9 – 1.2	0.2 – 0.5 0.3 – 0.2	0.8 – 0.5 0.7 – 0.8
Methanol fraction					1.2 – 1.3 1.0 – 1.0	0.2 –0.3 1.1 – 0.9
Leaf fractions						
Hexane fraction						
chloroform:methanol fraction(1:1)				0.4 – 0.3 0.3 – 0.3	0.6 – 0.7 0.8 – 0.6	
Methanol fraction	0.6 – 0.6	0.2 - 0.3 0.6 – 0.7	0.3 – 0.3 1.2 – 0.8	0.4 – 0.7 0.5 – 0.2	NG	

NG: no growth

Table 14: Antimicrobial activities of tisanes (Test concentration: 32 mg/ml).

Tisanes (32 mg/ml)	***E.coli***	***P.aeruginosa***	***S.typhimerium***	***S.aureus***	***A.niger***	***C.albicans***
Antidesma madagascariensis	0.8 – 0.6 0.6 – 0.7	NG	NG	NG	1.3 – 1.5 1.1 – 0.9	1.3 – 0.5 1.2 – 0.5
Ocimum tenuiflorum	0.6 – 0.9 0.9 – 0.7	0.4 – 0.3 0.7 – 0.8	1.0 – 1.1 1.3 – 1.3	NG	0.6 – 0.6 0.5 – 0.6	0.7 – 0.8 0.8 – 0.9
Plecthranthus amboinicus	1.1 – 1.2 0.5 – 0.5	0.4 – 0.6 1.0 – 0.8	0.6 – 0.8 1.0 – 1.0	0.5 – 0.5 1.2 – 0.7	NG	0.3 – 0.3 0.5 –0.3
Toddalia asiatica	0.2 – 0.3 0.4 – 0.6	NG	NG	0.7 – 0.2 0.6 – 0.4	NG	NG
Vepris lanceolata	0.8 – 0.3 0.7 – 0.4	0.6 – 0.4 0.8 – 0.6	0.3 – 0.4 0.6 – 0.5	NG	NG	0.2 – 0.2 0.4 – 0.5
Cassia fistula	NG	0.5 – 0.5 0.6 – 0.5	NG	NG	1.4 – 1.4 0.6 – 0.7	0.7 – 0.8 0.5 –1.1
Senna alata	0.6 – 0.5 0.8 – 0.8	0.5 – 0.4 0.7 – 0.4	0.5 – 0.6 0.8 – 1.0	NG	0.2 – 0.3 0.3 – 0.3	0.8 – 0.7 0.3 – 0.4
Solanum nigrum	0.5 – 0.6 0.3 – 0.4	0.6 – 0.4 0.4 – 0.3	0.5 – 0.5 0.7 – 0.5	0.5 – 0.4 0.3 – 0.4	0.8 – 0.8 0.2 –0.3	0.7 – 0.6 0.4 – 0.8
Cinnamomum camphora	0.8 – 0.7 0.7 – 0.8	0.8 – 0.5 0.5 – 0.6	0.4 – 0.4 0.7 – 0.5	0.4 – 0.5 0.4 – 0.3	0.7 – 0.2 0.5 – 0.7	0.9 – 0.9 0.9 – 0.8
Tristemma mauritianum	NG	NG	NG	NG	1.0 – 0.5 0.3 – 0.5	0.2 – 0.6 0.4 – 0.6

Table 14: Phytochemical screening of different extracts of *Antidesma madagascariense*

Plant extracts	**alkaloids**	**phenols**	**tannins**	**terpenes**	**saponins**	**Anthra quinones**	**coumarins**	**flavones and related flavo glycosides**
Leaf and Stem(crude water extract)	-	+	+	+	-	-	-	-
Stem fractions								
Hexane fraction	-	+	-	+	+	-	-	-
Chloroform:methanol (1:1) fraction	+	+	+	-	-	-	-	+
Methanol fraction	-	+	+	+	-	-	-	-
Leaf fractions								
Hexane fraction	-	-	-	+	-	-	-	-
Chloroform:methanol (1:1) fraction	-	+	+	+	-	-	-	-
Methanol fraction	-	-	+	-	-	-	-	-

Table 15: Phytochemical screening of different extracts of *Faujasiopsis flexuosa*

Plant extracts	**alkaloids**	**phenols**	**tannins**	**terpenes**	**saponins**	**anthraquinones**	**coumarins**	**flavones and related flavo glycosides**
Leaf and Stem (crude water extract)	-	-	+	+	-	-	-	+
Stem fractions								
Hexane fraction	-	-	+	+	-	-	-	-
Methanol fraction	-	-	+	+	-	-	-	-
Leaf fractions								
Hexane fraction	-	-	-	-	-	-	-	-
Chloroform: Methanol (1:1) fraction	-	-	+	+	+	-	-	-
Methanol fraction	-	-	-	+	-	-	-	-

Table 16: Phytochemical screening of different extracts of *Toddalia asiatica*

Plant extracts	**alkaloids**	**phenols**	**tannins**	**terpenes**	**saponins**	**anthraquinones**	**coumarins**	**flavones and related flavonoid glycosides**
Leaf and Stem (crude water extract)	-	-	+	+	-	-	-	-
Stem fractions								
Hexane fraction	-	-	+	-	-	-	+	-
chloroform:methanol (1:1) fraction	+	-	+	+	-	-	-	-
Methanol fraction	-	-	+	-	-	-	-	-
Leaf fractions								
Hexane fraction	-	-	+	-	-	-	-	-
Chloroform:Methanol (1:1) fraction	+	-	+	+	-	-	-	-
Methanol fraction	-	-	+	-	-	-	-	-

Table 17: Phytochemical screening of different extracts of *Vepris lanceolata*

Plant extracts	**alkaloids**	**phenols**	**tannins**	**terpenes**	**saponins**	**anthraquinones**	**coumarins**	**flavon and relate flavon glyco**
Leaf and Stem (crude water extract)	+	+	+	-	-	-	-	
Stem fractions								
Hexane fraction	-	-	+	+	+	-	-	
chloroform:methanol (1:1) fraction	+	-	+	-	-	-	-	
Methanol fraction	+	-	+	-	-	-	-	
Leaf fractions								
Hexane fraction	-	-	+	+	-	-	-	
Chloroform:methanol(1:1) fraction	+	-	+	-	-	-	-	
Methanol fraction	-	-	+	-	-	-	-	

Table 18: Phytochemical screening of *Senna alata*

Plant extracts	alkaloids	phenols	tannins	terpenes	saponins	anthraquinones	coumarins	flavone related flavono glycosi
Leaf and Stem(crude water extract)	-	-	+	-	+	+	-	-

Table 19: Phytochemical screening of *Cassia fistula*

Plant extracts	alkaloids	phenols	tannins	terpenes	saponins	Anthra quinones	coumarins	flavones a related flavonoid glycosides
Fruit pulp (crude water extract)	-	-	+	-	+	+	-	-

Table 20: Phytochemical screening of *Ocimum tenuiflorum*

Plant extracts	alkaloids	phenols	tannins	terpenes	saponins	Anthra quinones	coumarins	flavo and relate flavo glyco
Leaf and Stem (crude water extract)	-	-	+	-	-	-	-	
Flower (crude water extract)	-	-	+	-	+	-	-	

+ : presence of compound
- : absence of compound

4.0 Results and Discussion.

Antimicrobial screening of the tisanes: All of the tisanes were made in water as per the method used by the lay people.

Antidesma madagascariensis tisane: Active against the following bacteria: *P. aeruginosa, S. typhimerium, S. aureus*. These data correlate very well with its traditional use as it is used to wash boils and it appears to accelerate the healing process.

Ocimum tenuiflorum: Active against only against *S. aureus.* This plant is used mainly against bloat and flatulence and not so much for its antibacterial properties.

Plectranthus amboinicus: Active against *Aspergillus niger*. Again this plant is not known for its antibacterial properties as it is used mainly used against asthma.

Toddalia asiatica: Active against *P. aeruginosa, S. typhimerium, Aspergillus niger* and *Candida albicans.* This plant is not only commonly used against pulmonary disorders but is also very popular against malarial fevers and common fevers in general. The wide bacterial activity range observed would justify this use.

V. lanceotata: Active against *S. aureus* and *A. niger*. This plant is mainly used against pulmonary infections and not so much for its antibacterial activity. Nonetheless, the ethnobotanical data for Rodrigues shows that the plant is also used to wash wounds.

Cassia fistula: Active against *E. coli, S. typhimerium, S. aureus*. This plant is mainly used as a laxative and also to expel intestinal worms. The antibacterial profile is very interesting.

Senna alata: Active against *S. aureus.* A bath in the leaf decoction is used to wash boils and scabies as well as other skin infections such as eczemas. It's activity against *S. aureus* is significant.

Solanum nigrum: Not active against any bacterium. Interestingly, this plant is mostly used for its hypertensive properties and there is no ethnobotanical data for its antibacterial uses.

Cinnamomum camphora: Not active against any bacterium. This plant is again mainly used to wash parts of the body suffering from rheumatismal pains and not so much for its antibacterial properties.

Tristemma mauritianum: Active *against E. coli, P. aeruginosa, S. typhimerium, S. aureus*. On the other hand, this plant is particularly known for its uses against diarrhoea, dysentery and against skin infections. The antibacterial profile of the plant gives credence to this traditional information

Antimicrobial screens of the indigenous/endemic plants:

The initial screening of the indigenous and endemic plants showed very promising results. 28 Extracts inhibited the growth of one or more micro-organisms. Only 3 extracts showed no activity at all. No plant was completely inactive either since all of the plants were responsible for at least one extract with activity.

The percentage of the extracts that inhibited the various bacteria were as follows:

E. coli:	29%
P. aeruginosa:	77%
S. typhimerium:	39%
S. aureus:	74%
A.niger:	52%

No extracts inhibited *C. albicans*. Thus from the above calculations, it showed that ¾ of the extracts inhibited *P. aeruginosa* and *S. aureus*. This could well be an indication of the tannin-based activity against these bacteria since tannins are very common in plants.

MIC Determination:

Distribution of the MIC's:

8 mg/ml:	32%
4 mg/ml:	18%
2 mg/ml:	18%
1 mg/ml:	26%
0,5 mg/ml:	1,5%

No extract was found to be active at a concentration of 0,25 mg/ml but interesting results were obtained for a MIC of 1 mg/ml. This illustrates that at even at fairly low concentrations, activity was obtained. This could help justify to some extent, the utilisation of these plants by the lay people in traditional medicine.

Screening for activity after tannin precipitation.

A fairly large number of the extracts still had some or full activity after the tannin precipitation. It was also interesting to note that some micro-organisms were more sensitive to tannins than others. The percentages of cases where microorganisms was still being inhibited after tannin removed and was found to be:

E. coli:	10%
P. aeruginosa:	57%
S. typhimerium:	8%
S. aureus:	45%
A. niger:	0%

From the above-mentioned data, it appears that there are great differences in the sensitivity towards tannins by the various micro-organisms. *P. aeruginosa* and *S. aureus* were clearly much less sensitive when it comes to tannins but were obviously more affected by the other compounds present in the crude extracts.

Thus the general conclusions from this exercise were that other bio-active substances are present in the plant extracts that still account for activity once the tannins have been removed.

Further studies would be warranted in order to find out more on the nature of these compound(s) especially as these plants are indigenous/endemic to Mauritius and the surrounding islands.

Effects of extracts on contraction and relaxation on rat ileal strip *in vitro*.

Vepris lanceolata.

A stem methanol fraction extract elicited sustainable contractile responses on rat intestinal strips as from 500 µl. However the same extract showed weaker and non-sustainable contractile responses on rat aorta *in-vitro*.

Faujasiopsis flexuosa.

Methanol extract as well as chloroform: methanol (1:1) fractions displayed strong sustainable contractile responses on rat aorta as well as ileal strips. However no significant contractile responses were noted with methanol fraction extracts from the leaves. However the stem methanol fraction displayed strong sustainable contractile responses as from an early dose of 100 µl on rat aorta. No effects were noted on the intestine.

Antidesma madagascariense

Typical responses were elicited by stem methanol fraction on rat aorta strips in vitro. Stem methanol extracts also showed potent contractile effects on the intestine.

***Toddalia asiatica* (tisane)**

Leaf methanol fraction showed strong sustainable contractile responses on rat aorta strips. No significant contractile responses were noted on the intestine. However the extract obtained from the chloroform: methanol fraction from the leaf had definite contractile properties on the rat ileal strips.

Senna alata* and *Ocimum tenuiflorum

Extracts from both tisanes did not show contractile properties on rat ileal strips.

5.0 Conclusions and recommendations.

Findings from this screening study provide important baseline data regarding possible biological properties of the indigenous plants. As far as we are aware, it is the tradition in Mauritius that for most ailments and symptoms, lay people still rely heavily on the use of tisanes and medicinal plants despite the fact that not much is known about the possible beneficial and side effects of the species being used.

Our findings highlight the risks of use or overuse or abuse of these plants. Though our work definitely showed that many indigenous plants do possess quite useful biological properties especially when it comes to the effects on the intestine (whereby the extracts could be used against diarrhoea), the properties displayed on the aorta should not be overlooked. Almost all the extracts screened did show contractile properties on the rat aorta strips to various degrees. Once again use of these plants by persons who may have coronary artery diseases should be discouraged. The reason being that though the intestinal properties are well appreciated, contraction on the aorta could lead to serious cardiac disorders such as angina.

Findings from this work form the basis of certain stages of Phase I screening studies for potential pharmacological studies and findings at this stage should be dealt with in

a very cautious manner. It would be advisable now that further studies are undertaken on these indigenous plants to establish relevant properties that could lead to their effective use by people.

5.1 Toxicity tests.

Toxicity tests of the various extracts carried out on a small number of rats have revealed that each extract tested has led to an increase in mean weight gained as compared to the controls. At this stage one has to be cautious in interpreting the data. No statistical analyses could be performed since tests were performed on too small a sample size. The possible mechanisms or reasons for the observed gain in weight in the animals are not clear yet.

Further work should in fact consider testing a smaller number of extracts that have caused a greater gain in body weight on a larger sample size of laboratory animals. Measurements in these proposed studies should also include determination of lipids levels by colorimetric methods using commercially available kits (to be adapted for small animals).

- Studies of the various extracts on the animals should be performed over a longer period of time.
- Effects of extracts on body weight should be also studied on other animals such as rabbits.
- Studies on the urinary excretion of metabolites could also be envisaged.

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ANNEX 1 : TRACINGS FROM THE IN VITRO ASSAYS:

- Tests performed on the rat intestine strips *in vitro*
- Tests performed on the rat aorta strips *in vitro*

Using extracts from the different plants:

- *Antidesma madagascariensis* (Euphorbiaceae)
- *Faujasiopsis flexuosus* (Asteraceae)
- *Vepris lanceolata* (Rutaceae)
- *Toddalia asiatica* (Rutaceae)
- *Senna alata* (Leguminosae)
- *Ocimum tenuiflorum* (Lamiaceae)