

The Pharmacological Properties of the Isolated Bioactive Compounds from Endemic Medicinal Plants of Mauritius

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Abstract

The antifungal, antibacterial as well as the ability to contract and relax toad and rate ileal strip by extracts obtained from endemic medicinal plants of Mauritius are reported. The plants tested are: *Antirhea borbonica* Gmel.; *Chassalia coriacea* Verdc. Var. *coriacea* (Rubiaceae), *Psiadia terebinthina* (Pers.) Voigt. (Asteraceae), *Canarium paniculatum* Lam. Benth. Ex Engl. (Burseraceae), and *Erythroxylum* spp. (Erythroxylaceae). The extracts of the plants from which the tannins have been removed are also tested. Attempts have also been made at isolating and characterising some of the bioactive components. The correlation of the ethnobotanical use with the presence of active components is discussed as well.

INTRODUCTION

Mauritius is a tropical island in the southwest Indian Ocean and has numerous plant resources. The people of Mauritius have a long standing tradition in the use of ethno-medicine and the practice of traditional medicine is still very strong in the treatment of minor ailments. The use of the flora, however, had been restricted mainly to exotic plants brought by immigrants from Africa, Madagascar, Asia and China nearly half a century ago. In a survey carried out on the traditional uses of plants both in Mauritius and Rodrigues, only a small percentage (approx. 5%) of the endemics were being used as medicinal plants (Fakim, 1990; Gurib-Fakim et al., 1995-1997).

It is well established that plant-derived compounds offer potential sources of pharmaceutical agents. A systematic study of the flora is currently being carried out in the search of new bioactive molecules emanating from endemic medicinal plants of Mauritius. Attempts are also being made at validating some of the ethno-botanical claims from the locally utilised endemic medicinal plants.

In order to ascertain the biological active compounds attempts have to be made for the isolation of the compounds and identification of the pharmacological properties.

MATERIALS AND METHODS

Plant Material and Extraction

The plants tested during the course of this work were collected in the native forests of Macchabé and Rivière Noire and were authenticated by the curator of the National Herbarium, Mauritius Sugar Industry Research Institute (MSIRI), Mr. J. Guého. A voucher specimen of each plant has been deposited at the Herbarium collection of the Faculty of Science, University of Mauritius.

The plant parts were dried and ground, then 15-20 g of the powder was extracted in about 200 ml of methanol. The extracts were dried *in vacuo*. The yield was calculated as g extract/g dry plant material. The extracts were dissolved in a mixture of methanol/sterilized water (1:1) with a concentration of 8 mg/ml. The methanol extract was also used for the isolation of the chemical components.

Testing Microbiological Activity

The extracts were tested against Gram positive (*Staphylococcus aureus*) and Gram

negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and fungi (*Candida albicans* and *Aspergillus niger*). The bacteria and fungi were clinical isolates obtained from the local hospital. The identity was confirmed by the microbiologist at the hospital. The extract of *A. niger* was obtained from the Microbiology department of the Faculty of Science of the University of Mauritius.

The program used for the screening is the agar dilution method described by Mitscher et al. (1972), designed for the evaluation of antimicrobial activity extracts of higher plants. Bacteria were incubated at 37°C for 24 hrs, fungi were incubated at 37°C for 48 hrs.

Tannins were removed from the extract by precipitation with gelatine prior to testing. The plant extracts, obtained from 10 g of the plant material, was dissolved in 25 ml of hot distilled water, stirred and allowed to cool at room temperature. After filtration, a 1% gelatine solution was added dropwise to the filtrate until no further precipitation was noticed. The mixture was centrifuged and the supernatant layer was evaporated to dryness resulting in a crude extract without tannins. The test concentration was then adjusted so that it was possible to compare the activity before and after the removal of the tannins.

In Vitro Pharmacological Trials

Sprague-Dawley rats weighing 50 to 100 g were sacrificed by a severe blow on the head. The abdominal cavity was opened and a stretch of the lower part of ileum was removed, cut free of mesentery and sectioned into pieces of 1 cm length. Each ileal strip was washed to remove any remaining food material using a hypodermic syringe filled with Krebs' solution. The strips were immersed in Krebs' solution (bubbled with carbogen, 95% O₂ and 5% CO₂) containing a few drops of heparin (5000 IU/L) to prevent any blood clot formation, until they were mounted in the organ bath one at a time according to the technique described by Gurib and Subratty (2001). Before mounting each strip, the glass-jacketed organ bath was filled with Krebs' solution (25 ml) and the latter was bubbled with carbogen for five minutes by Subratty & Moonsamy (1998). The organ bath solution was maintained at 37°C with a thermostatted water pump (Lauda, model-1) and the pH of the solution was adjusted to 7.4. Mounting of strip in the organ bath involved inserting two triangular stainless steel hooks into the lumen enabling the strip to be held horizontally in the bath. One of the triangular hooks was pinned to a fixed point in the apparatus and the other one was connected to a force-displacement transducer (Model LB-5, Showa Shokki, Japan). Contractile and relaxation responses were measured isometrically with the transducer and were recorded on a multipen recorder (Rikadenki Model R50, Japan). After mounting, each organ strip was adjusted to a resting tension of 1.5 g and was allowed to equilibrate in the organ bath for about 15 mins until a baseline tone was achieved before being challenged with the plant extract under test.

After stabilization, each mounted strip was challenged with 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 µL of the test plant extract. After each 100 µL addition, the organ strip was allowed to give the maximum response before the next addition. Contractile responses were recorded as increases in the baseline tone while relaxation responses were recorded as decreases in the baseline tone taking into account the fact that during stabilization, a tension of about 1.5 g was already applied on the strips.

For challenging organ strips in vitro, dried plant extract (132 mg) was dissolved in methanol (1 ml) (aqueous plant extracts were dissolved in water). Addition of this solution (100 µL) to the organ bath already containing Krebs' solution (25 ml) afforded a final bath concentration (FBC) of 0.528 mg/ml or 528 ppm plant extract in the organ bath.

For each series of experiments, a control strip was included and challenged either with methanol (100 µL addition) or water (100 µL addition) depending on what solvent was used to dissolve the plant extract.

RESULTS AND DISCUSSION

The indigenous/endemic plants, which have been tested by us and discussed in this paper were as follows: *Antirhea borbonica* Gmel., *Chassalia coriacea* Verdc. var.

coriaceae (Rubiaceae), *Psiadia terebinthina* (Pers.) Voigt (Asteraceae), *Erythroxylum* spp. (Erythroxylaceae).

***Antirhea borbonica* Gmel. (Rubiaceae)**

The plant is considered to be rare. The leaf and bark decoction is used locally against chronic diarrhoea and dysentery (Gurib-Fakim et al., 1997). The green fruit extract showed weak relaxation of the pre-contracted strips of toad ileum. The crude methanol extracts obtained from the leaves manifested activity against *P. aeruginosa* and *S. aureus* at a concentration of 8 mg/ml. The methanol extract of the unripe fruit is also active against *A. niger* also at a concentration of 8 mg/ml.

One new diterpene and one new sesquiterpene were isolated and characterised phytochemically, the structures of these compounds are being examined.

***Chassalia coriacea* Verdc. var. *coriacea* (Rubiaceae)**

This endemic plant is not threatened. The plant is reported to be used as an astringent in the local pharmacopoeia. Previous work has shown that the crude methanolic extract of the leaves showed severe contraction on the smooth ileal toad muscles and also exhibited anti-microbial activities against *P. aeruginosa* (Pedersen et al., 1999). These observations on the astringency of the plant has to some extent been confirmed by the activity of the leaf extract in its action against the isolated intestine strip, notwithstanding the fact that its an important criterion in the treatment of infectious diseases such as diarrhoea and dysentery (Marie, 2000).

In our investigation the leaf extracts manifest antimicrobial activity against both *Pseudomonas aeruginosa* and *Aspergillus niger* at a concentration of 8 mg/ml in both cases. The leaf extract also showed sustainable and cumulative type of contractile responses on non-precontracted ileal strips. Such contractions are usually observed when smooth muscles are challenged with pharmacological agents such as acetylcholine. Further work is being undertaken to establish dose-response effects of the extract on smooth muscle in vivo.

The methanol fraction has yielded quercetin-3-O-glucose rhamnoside and the structure has been confirmed by mass spectroscopy.

***Psiadia terebinthina* (Pers.) Voigt (Asteraceae)**

The plant is not threatened. The leaf decoction is used asthma and fever while the leaf poultice is applied on abscess and boils (Gurib-Fakim et al., 1995).

The crude methanol extract of the leaf showed activity against *E. coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* at a concentration varying from 8 mg to 1 mg/ml. The fractionated extracts failed to relax carbachol contracted guinea pigs tracheal rings.

Its chemical constituent kaemferol-3-methyl ether was characterised by NMR and mass spectroscopy. It showed sustained antibacterial activity against *E. coli*, in vitro, at a concentration of 25 mg/ml. Another compound isolated from the methanol fraction of this plant is quercetin-3-methyl ether. The latter showed sustained activity against *Escherichia coli*, in vitro, at a concentration of 50 mg/ml.

From the same extract, another flavonoid was isolated, namely kaemferol-3,7-dimethyl ether. This compound however did not show any sustained activity against any of the bacteria tested.

Both *Psiadia terebinthina* (and *P. viscosa* as well) contain 3-caffeoyl quinic acid (chlorogenic acid) and 3,4-dicaffeoyl quinic acid. Both compounds are also reported to inhibit the myeloperoxidase enzyme.

A new compound has been isolated from this same species and it has been characterised as being: 5-(2-furan-3-yl-ethyl)-5,6,8a-trimethyl-3,4,4a,5,6,7,8,8a-octahydronaphthalene-1-carbaldehyde. The latter has been shown to show anti-fungal activities against *Cladosporium cucumerinum*.

Psiadia species are commonly used in Mauritius as expectorants and against

asthma. As mentioned earlier on, the fractionated leaf extracts failed to relax carbachol contracted guinea pigs tracheal rings. Nonetheless, 3-caffeoyl quinic acid and 3,4-dicaffeoyl quinic acid have been isolated and identified. In the literature, it has been reported that tetragalloyl quinic acid is the major anti-asthmatic principle isolated from *Galphimia glauca* (Malpighiaceae). This component showed highest activity when albumin was used as the bronchoconstricting agent. Yet the only major difference between tetragalloyl quinic acid and caffeoyl quinic acid are the presence of a double bond and one OH group on the galloyl moiety in tetragalloylquinic acid and which are absent in the caffeoyl moiety in caffeoyl quinic acid. Also tetragalloyl quinic acid has 4 galloyl groups attached to the quinic acid nucleus. In the caffeoyl derivatives, there are one and two caffeoyl moieties respectively attached to the quinic acid nucleus in 3-O-caffeoyl and 3,4-O-dicaffeoyl quinic acid. It may be extrapolated that the major ingredients possibly responsible for the anti-asthma activity of *P. terebinthina* are the caffeoyl quinic acid derivatives. Further tests need to be carried out in order to ascertain this observation.

***Canarium paniculatum* (Lam.) Benth. ex Engl. (Burseraceae)**

The plant is considered locally as a highly endangered species. The leaf poultice and the resin are applied on parts of the body suffering from rheumatism and it also treats ulcerations of the skin. The crude extracts of the stem show activity against *E. coli* while that of the wood show activity against *E. coli*, *P. aeruginosa*, *S. typhi* at an initial concentration of 8 mg/ml. The fractionated extract of the bark was again tested. The less polar fractions showed the greatest activity. The hexane, chloroform and chloroform/methanol extracts showed activity against all four mentioned bacteria. The methanol extract was inactive. The crude methanol extracts of the leaf and bark showed contraction of toad smooth ileal strips (Marie, 2000).

From the leaves, (-)-epicatechin has been isolated. The bark has given rise to 3-O-galloylepigallocatechin while the wood has yielded methyl gallate ester, gallic acid and penta galloyl glucose. The resin has yielded methyl gallate ester as well as amyrin (Marie, 2000).

***Erythroxyllum* spp. (Erythroxylaceae)**

The stem and bark decoction is used against kidney stones. The leaves mixed with other plants are used against fever. The whole plant is considered to be useful against nephritic colic. Four species of the endemic *Erythroxyllum* genus have been tested and they are: *E. laurifolium*, *E. hypericifolium*, *E. macrocarpum* and *E. sideroxyloides*. The methanol fraction from the fractionated extracts of the leaf and stem have been shown to manifest antibacterial properties against all of the tested bacteria. The minimum inhibitory concentration (MIC) was found to be 8 mg/ml. However, the aqueous fraction of *E. macrocarpum* was the only extract effective in all of the tested bacteria with a MIC of 1 mg/ml.

Further pharmacological tests performed on the toad and rat ileal strips, the following observations were made on the leaf methanol extracts of the plants: *E. sideroxyloides* showed sustained contractile responses on toad and rat ileal strips. For the rat tracheal and aortal strips, the methanol leaf extracts showed slight relaxation followed by contractile responses and a biphasic response – contraction followed by relaxation respectively.

E. hypericifolium only showed relaxation responses followed by sustainable contraction on toad ileal strip. The methanol leaf extract showed weak contractile responses against rat tracheal strip and sustainable contraction against rat aortal strip.

The methanol leaf extract of *E. laurifolium* showed contraction leading to tetanus. Biphasic response was observed on rat ileal strips. The extract also showed biphasic responses – relaxation followed by contraction on rat tracheal strip and relaxation on rat aortal strip.

E. macrocarpum leaf extracts showed sustainable contraction leading to tetanus

and with no significant response on rat ileal strip. The leaf extract showed biphasic responses (relaxation followed by contraction) both for rat tracheal strip and aortal strips.

The leaves of all of the above-mentioned species are reported to contain the flavonoids quercitrin (quercetin-3-O-rhamnoside), isoquercitrin (quercetin-3-O-glucoside) and (+)-catechin.

The work done on the species of the Erythroxyllum lend support to the observations made by Hegnauer (1981) when he reported that the major flavonoids of the Erythroxyllaceae family were flavonols like quercetin and its glycosides.

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