

Kolorex®

yeast free living!



## Scientific Summary

### Table of Contents

Horopito - the ancient herb.....	2
Traditional Use.....	2
Anti- <i>Candida</i> Discovery .....	2
RedLeaf® Horopito .....	3
Antifungal and Antibacterial Activity .....	3
Mode of Action.....	4
Toxicology and Mutagenicity.....	4
Safety.....	4
<i>in vitro</i> Efficacy .....	5
<i>in vivo</i> Studies.....	9
Stability Studies.....	10
Clinical Studies.....	11
Kolorex™ Products .....	12
References .....	12

### Horopito – the ancient herb



Horopito (*Pseudowintera colorata*) only grows in New Zealand. This ancient shrub is a member of the primitive Winteraceae family, common to the Southern Pacific. It has features of the earliest evolved flowering plants, and appears in the fossil record over 65 million years ago. It is a very slow growing plant that lacks the specialist water conducting tubes found in nearly all other flowering plants. It grows well only in damp areas, especially under temperate rain Forest.<sup>1</sup>

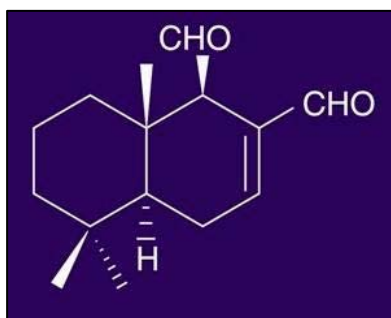
### Traditional Use

Horopito has a long history of medicinal use by New Zealand's indigenous Maori population. The leaves were bruised, steeped in water and used for paipai (a skin disease), and venereal diseases. The leaves were chewed for toothache<sup>2</sup>. It was also used for skin diseases like ringworm. A decoction of the leaves was often used to allay inward pain and is honoured with the name 'Maori painkiller'<sup>3</sup>.

### Anti-*Candida* Discovery

In 1982 Professor J.R.L. Walker and his team at New Zealand's University of Canterbury isolated a sesquiterpene dialdehyde called polygodial in the leaves of Horopito (see Figure 1).

Figure 1. Chemical structure of Polygodial



They demonstrated that polygodial had strong anti-fungal activity against the yeast *Candida albicans*. They compared its activity with that of Amphotericin B (a proprietary pharmaceutical product used to treat systemic mycoses). The polygodial extract from Horopito gave larger zones of inhibition against *C. albicans* and was effective from day one whereas the inhibitory effect of Amphotericin B against *C. albicans* required three to four days incubation to become effective<sup>4</sup>. See Table 1

for test results.

## Scientific Summary

Table 1. Comparison of antibiotic activity of polygodial and Amphotericin B against *C. albicans*. Inhibition expressed as mean diameter (mm) of zone of inhibition<sup>4</sup>

Concentration (ug/disc)	Time (days)				
	1	2	4	8	11
<b>Polygodial</b>					
10	17.1	16.5	15.2	14.7	14.4
5	15.0	13.4	11.2	11.5	10.5
1	9.0	8.0	7.3	7.0	7.0
<b>Amphotericin B</b>					
100	F	F	8.2	8.3	8.3
10	F	F	6.3	6.5	6.4
1	F	X	X	X	X

F = Faint zone of inhibition; x = no inhibition

### RedLeaf® Horopito

The major constituents of Kolorex Horopito leaves are 13 terpenes, 8 sesquiterpenes (of which polygodial is dominant) and at least 4 flavonoids<sup>5</sup>.



Horopito grows wild through much of New Zealand's elevated and high rainfall regions but varies in appearance and growth habit. Forest Herbs Research Ltd sampled and compared all the major population groups. The samples were extracted and assayed for polygodial content and for their effectiveness against *C. albicans*. There was a five-fold difference between the most active and least active subspecies<sup>6</sup>. RedLeaf Horopito is derived from only the most active plants.

### Antifungal and Antibacterial Activity

In 1998 Kubo's team demonstrated that polygodial had strong antifungal activity against the yeast like fungi *C. albicans*, *C. utilis*, *C. krusei*, *Cryptococcus neoformans*, *S. cerevisiae* and also filamentous fungi including *T. mentogrophytes*, *T. ruburum* and *Penicillium marneffeii*. Polygodial's antifungal activity was strongly increased under acidic conditions. Unlike Amphotericin B, polygodial did not show any hemolytic activity<sup>7</sup>.

In the area of food preservation polygodial exhibits synergistic fungicidal properties. Sorbic acid is considered one of the least harmful preservatives in use but high concentrations are necessary for fungicidal activity. The fungicidal activity of sorbic acid against *S. cerevisiae* was enhanced 64 fold and that of benzoic acid 400 fold when these common preservatives were combined with half the minimum fungicidal concentration of polygodial<sup>8</sup>. EDTA is another food

## Scientific Summary

preservative upon which polygodial exerts a synergistic effect, presumably by facilitating its transport into yeast cells<sup>9</sup>. *Zygosaccharomyces baillii* is a spoilage yeast that can survive in acid media with ethanol such as wine, however polygodial controls it at very low concentrations<sup>10</sup>.

In addition to its antifungal activities, polygodial has moderate antibacterial activity against both gram positive bacteria (including *Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (including *Escherichia coli* and *Salmonella choleraesuis*) with minimum bactericidal concentrations ranging from 100–400ug/ml<sup>11</sup>.

### Mode of Action

Using *S. cerevisiae* as a model, polygodial was found to act as an antifungal in several ways. Polygodial's primary antifungal action is as a nonionic surfactant, disrupting the lipid-protein interface of integral proteins nonspecifically, denaturing their functional conformation. It is also likely that polygodial permeates by passive diffusion across the plasma membrane, and once inside the cells may react with a variety of intracellular compounds<sup>12</sup>.

### Toxicology and Mutagenicity

Toxicological assessment of Kolorex Horopito & Aniseed capsules (175mg of each) indicates that this formula is not toxic following acute exposure up to the level of 2 grams per kg bodyweight. At this dosage satisfactory body weight gains were maintained and macroscopic examination of the abdominal and thoracic cavities revealed no abnormalities<sup>13</sup>.

In contrast to compounds of a similar structure with strong biological activity, polygodial has been shown to be nonmutagenic (Ames and V79/HGPRT assay), and exhibit the least cytotoxicity<sup>13</sup>.

Plants containing the main active ingredient in Horopito (polygodial) have been traditionally used as foods or medicines in Japan, South America and Africa. There is no historical evidence of toxicity of Horopito by either oral ingestion or topical application.

### Safety

Approximately one million Kolorex capsules containing milled Horopito have been sold annually per year for the last thirteen years. Over this time Forest Herbs Research has documented fourteen adverse reactions. Half of these relate to nausea or vomiting and half to rashes. Over the last two years about one million Kolorex soft gel capsules containing Horopito extract have been sold. Only three adverse reactions of transient nausea have been reported.

Kolorex Intimate Care cream has been on the market for 20 years, during which seven incidents of adverse reactions have been reported of which two were severe allergic reactions. No adverse reactions have been reported for Kolorex Foot & Toe Cream.

## Scientific Summary

Although there is no evidence of tetragenicity, as a precaution it is suggested that pregnant women and small children do not take the oral formulations.

### *in vitro* Efficacy

#### *Kolorex Horopito leaf*

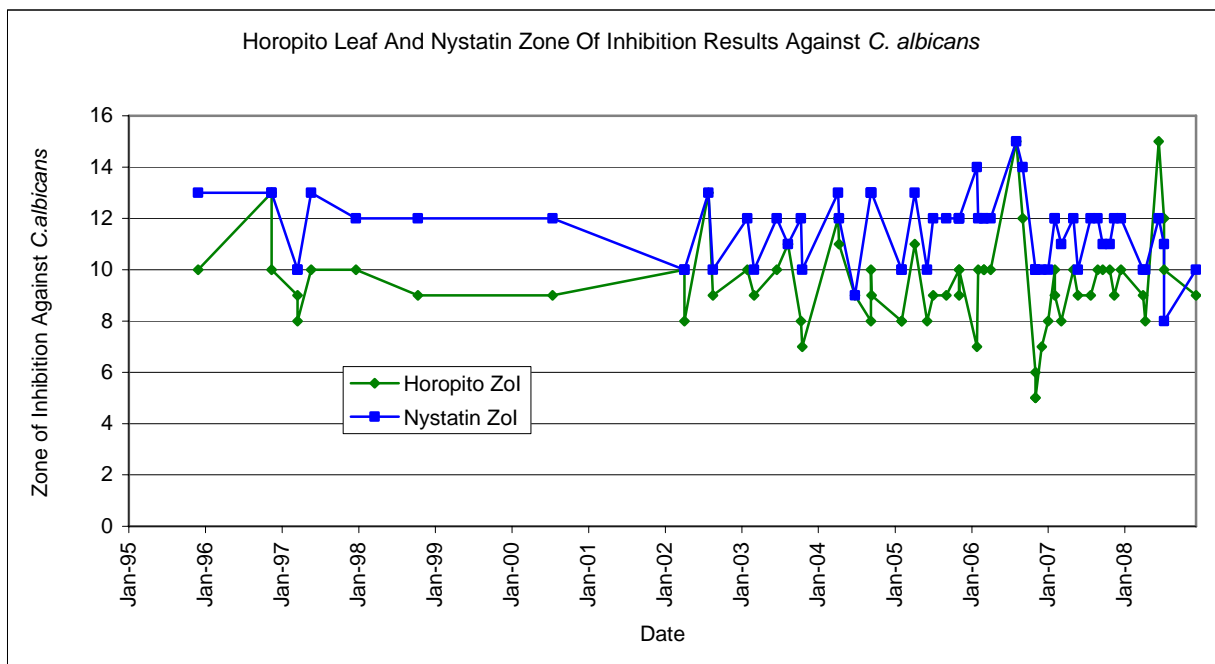
Each batch of Horopito leaves is independently assayed and must reach a specified level of anti *Candida* activity before it is used in Kolorex products.

Figure 2. Zone of inhibition against *C. albicans* demonstrated by the clear area around discs that have been impregnated with anti *Candida* agents.



Historical leaf testing results for the inhibitory activity of Horopito leaf samples against *C. albicans* compared with the antifungal treatment drug Nystatin are shown in Figure 3.

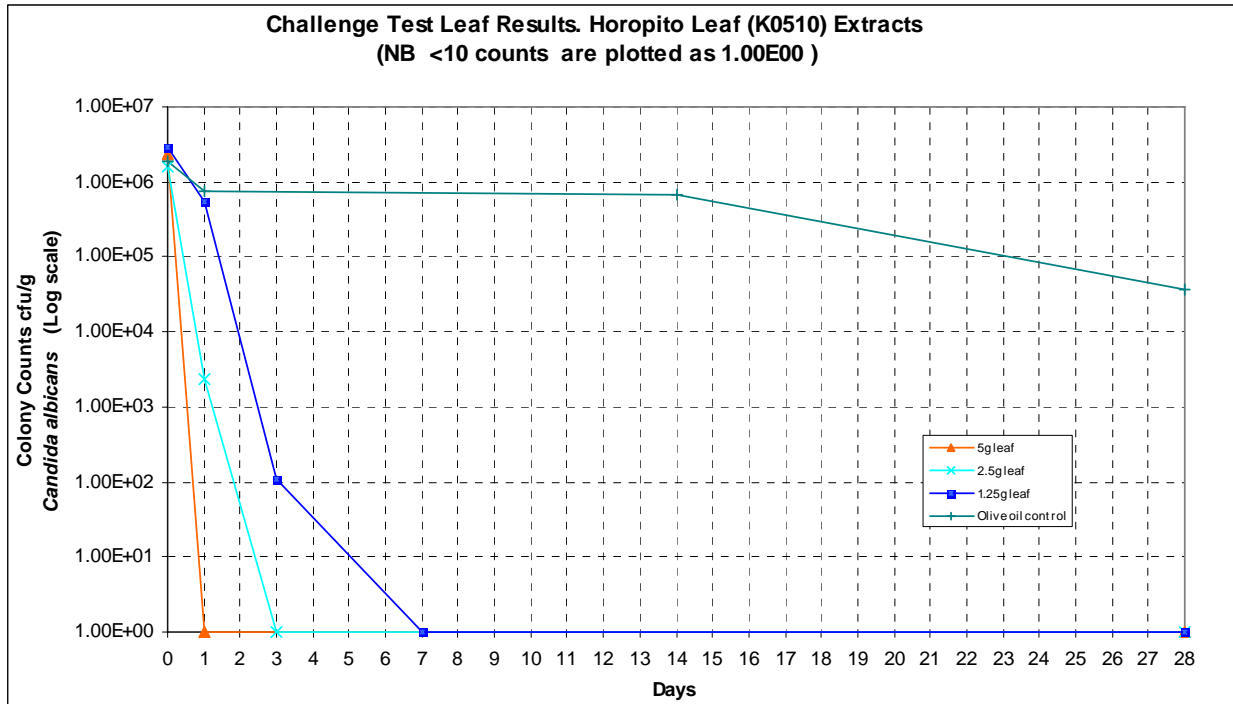
Figure 3. Horopito Leaf Zone of Inhibition (Zol) Test Results<sup>14</sup>



## Scientific Summary

Zone of Inhibition testing was stopped after the University of Canterbury discontinued its testing service. A new antifungal test based on the challenge test (European Pharmacopoeia General Text 5.1.3) was developed to monitor the antifungal capacity of Horopito leaf used in Kolorex products. The antifungal efficacy of the infusion can be seen in Figure 4, where the *C. albicans* colonies are quickly reduced to zero<sup>15</sup>.

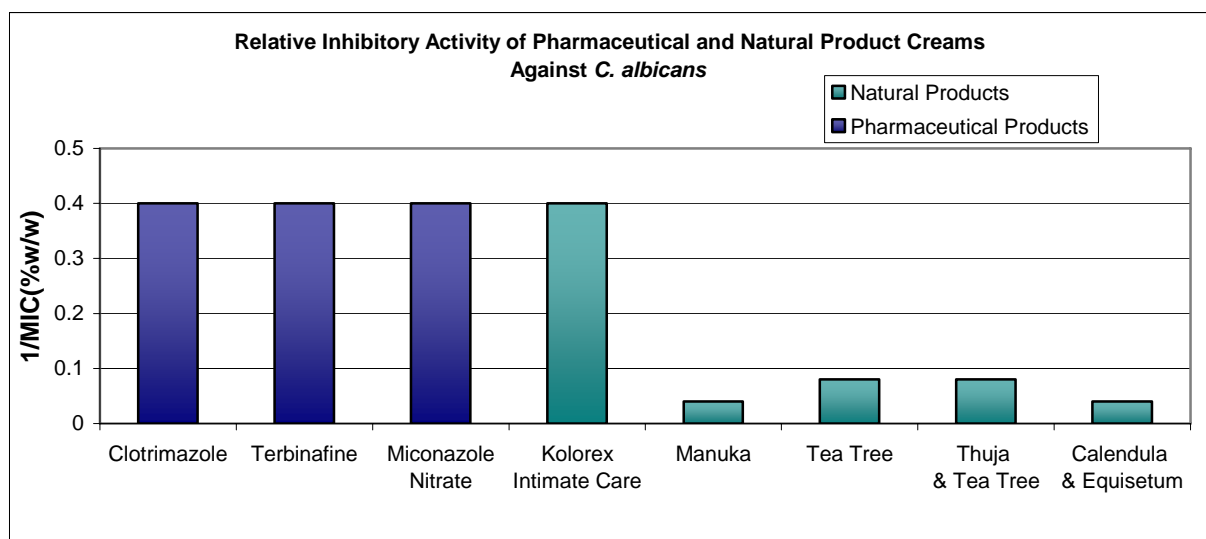
Figure 4. Challenge test results for various concentrations of Horopito Leaf Extracts



### Kolorex Intimate Care Cream

Kolorex Intimate Care Cream is more effective than other natural products and as effective as the pharmaceutical products tested (see Figure 5).

Figure 5. Kolorex Intimate Care Cream inhibitory activity compared with other products<sup>16</sup>.

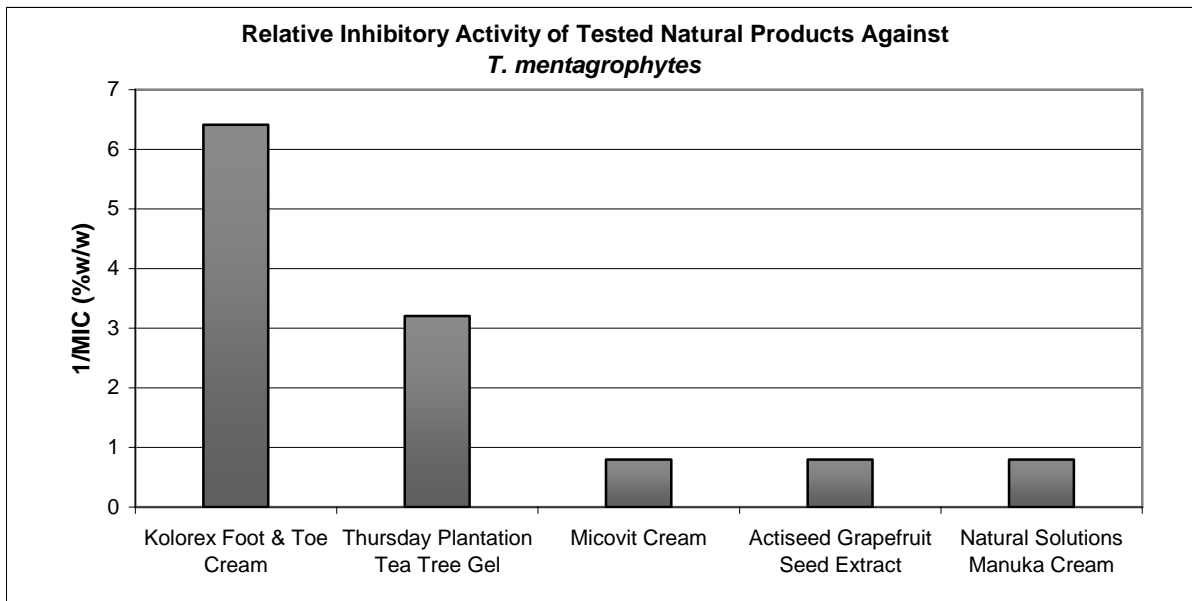


## Scientific Summary

### *Kolorex Foot & Toe Cream*

The testing of Kolorex Foot & Toe cream (See Figure 6 below) demonstrates that it has higher antifungal activity against one of the main athlete foot fungi (*T. mentagrophytes*), than any other natural product that was tested<sup>17</sup>.

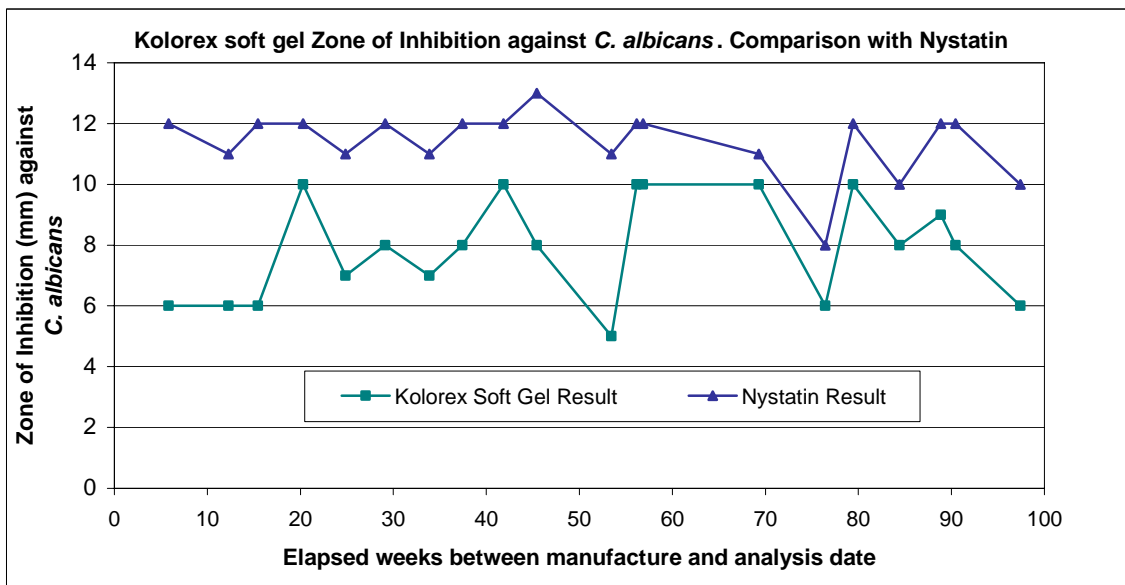
Figure 6. Relative inhibitory activity against *T. mentagrophytes* of Kolorex Foot & Toe Cream compared to other natural products tested.



### *Kolorex Advanced Intestinal Care soft gels*

The first production batch of Kolorex softgels was assayed for its effectiveness against *Candida albicans* on a monthly basis for almost two years, using the pharmaceutical anti-yeast product Nystatin as a control. See Figure 7. After 94 weeks the softgels are inhibiting the growth of *C. albicans* as effectively as when they were manufactured<sup>18</sup>.

Figure 7. Kolorex soft gel Zone of Inhibition against *C. albicans* test results compared with Nystatin



## Scientific Summary

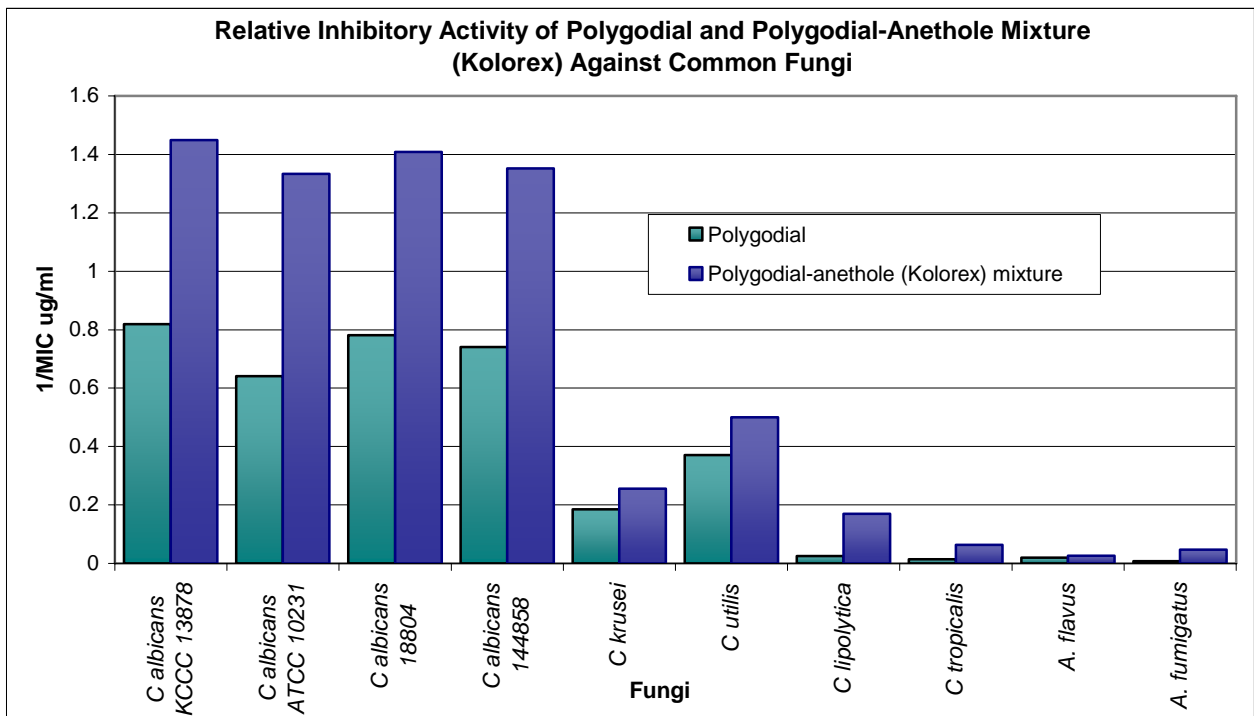
### *Kolorex Horopito & Aniseed Capsules*

Kolorex Horopito & Aniseed capsules exhibit a wide profile of antifungal activity. This discovery by a group of researchers led by Metugriachuk<sup>19</sup> found that the remarkable in vitro antifungal activity of the Kolorex mixture was better than polygodial alone (as illustrated in Figure 8). Furthermore they found that there was no significant toxicity associated with Kolorex. The most commonly used antibiotics however, do have the disadvantage of toxic side-effects. Their study concluded that “Kolorex is ... expected to be a promising compound for the development of therapeutic regimens acting through a synergistic effect”.



Photo: RedLeaf® Horopito plants at Forest Herbs Research Organic Farm

Figure 8. Relative activity of polygodial and polygodial-anethole mixture against common fungi<sup>19</sup>



Recent research using a Horopito and aniseed mixture containing 50mg ground Horopito (*P. colorata*), 41.5mg Aniseed (*P. anisum*), 2.9mg *L. acidophilus* and 5.6mg vitamin C was tested on patients with operated gastrointestinal cancer. The patients had a history of ongoing candiduria

## Scientific Summary

(urinary candida infection). It was found that a significant percentage developed urinary *Candida* infections following chemotherapeutic treatment. The Horopito formulation was used without any side effects and was successful in treating the infections in the majority of cases. The researchers recommend this natural antifungal phytochemical and think consideration should be given for its use as a prophylactic<sup>20</sup>.

### Note:

Forest Herbs Research considers Horopito as the active ingredient and aniseed as an excipient based primarily on its flavour and digestion aiding properties. If anethole from the aniseed exerts a synergistic effect this is an additional benefit but not relied upon in the formulation.

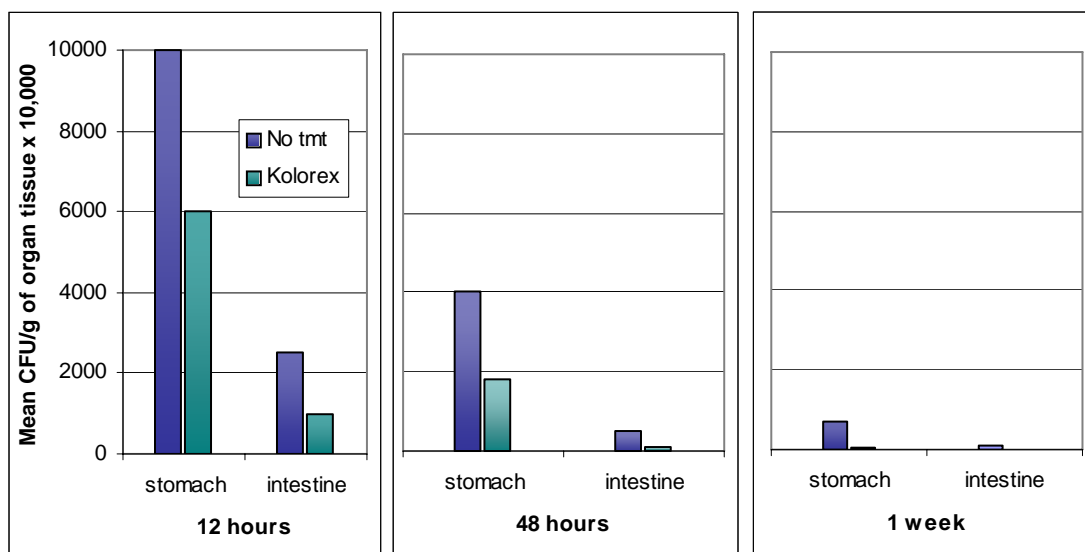
### Kolorex Mouth & Throat Care Tea

Results from research by Nakajima and colleagues<sup>21</sup> indicate that a Kolorex product (containing a polygodial and anethole combination) applied to the oral cavity inhibits the growth of different *Candida* yeasts. The combination of Horopito leaf and aniseed used in Kolorex Mouth and Throat Tea is designed for the cleansing of mucosal surfaces.

### *in vivo* Studies

In 2001 Naito and his team<sup>22</sup> investigated the ability of a Kolorex product containing polygodial and anethole to protect the gut of mice from colonization and dissemination of *Candida albicans*. After mice were inoculated with *C. albicans* and treated with Kolorex, testing of intestinal samples showed that Kolorex treated mice had a much reduced concentration of *C. albicans* per gram of tissue (see Figure 9). The data suggested that the Kolorex product might exert an early competitive effect against colonisation. Chemically synthesised antifungal treatments have been devised but they have the possible drawbacks of toxic effects and bacterial overgrowth. With the natural Kolorex product no toxicity or bacterial dissemination occurred during the observation period.

Figure 9. *C. albicans* concentration in tissue tested: Effect of 48hr pre-treatment with Kolorex<sup>22</sup>



## Scientific Summary

Further research by Marotta and associates<sup>23</sup> in 2006 replicated the effect of Kolorex on reducing the concentration of *C. albicans* in organs inoculated with the fungus. Their research was carried out using conditions of protein-calorie malnutrition often experienced by the elderly. It showed that Kolorex treatment significantly decreased the absolute number of organs infected and enabled complete clearance of *C. albicans* in the lungs. The study therefore concluded that Kolorex exhibited potential clinical interest for specific conditions of calorie-protein malnutrition.

An *ex vivo* study by Nakajima<sup>24</sup> who used a Horopito and aniseed mixture to inhibit the growth of *C. albicans* in the oral cavity has already been mentioned in connection with Kolorex Mouth & Throat Care Tea. This research concluded that in contrast to the commonly used oral antiseptics containing chlorhexidine, the antifungal action of Kolorex was more constant against all species tested (including *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. guillemontii*, *C. parapsilosis* and *C. krusei*) with a minimum inhibitory concentration of 1:20 (diluted with sterilised distilled water) of Kolorex.

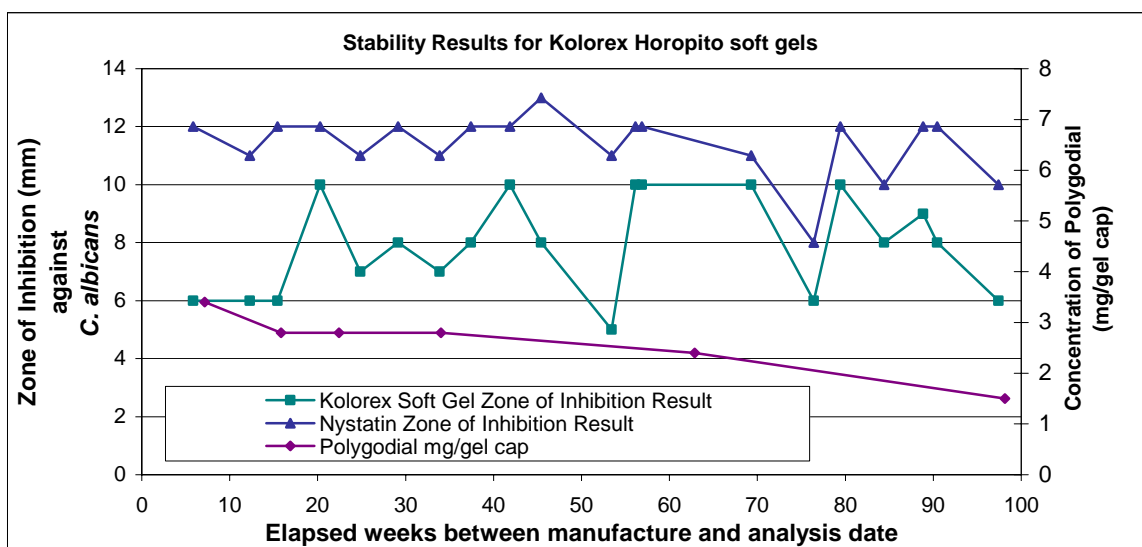
### Stability Studies

#### *Kolorex Horopito soft gels*

The softgels are as effective *in vitro* against *Candida albicans* at 94 weeks, as they were immediately after manufacture<sup>25</sup> (see zone of inhibition results Figure 10).

The stability of the active ingredient, polygodial, is being studied under controlled storage conditions by Southern Cross University, Australia. These results are graphed in Figure 10.

Figure 10. Kolorex Advanced Intestinal Care 500mg Soft Gel Capsule Stability Over 100 Weeks. Zone of Inhibition Results against *C. albicans* compared with Nystatin and Concentration of Polygodial (mg/gel cap)



### Clinical Studies

#### *Kolorex Herbal Capsules*

An open study conducted by eleven NZ naturopaths in 1992 for Forest Herbs Research examined the therapeutic effect of capsules containing milled Horopito (300mg) in patients diagnosed with chronic intestinal candidiasis. This study demonstrated a symptom improvement rate in 76% of cases<sup>26</sup>.

In 1997 the Pavlodar City Centre for Clinical Immunology and Reproduction carried out a study on patients diagnosed with chronic recurring intestinal candidiasis. It compared 22 patients taking capsules containing milled Horopito (300mg) with 10 patients administered fluconazole (Diflucan, Pfizer). All patients taking fluconazole and 90% of patients taking Kolorex capsules showed a significant improvement after 7 and 14 days respectively<sup>27</sup>.

#### *Kolorex Intimate Care Cream*

A clinical study on the efficacy of Kolorex Intimate Care cream against relapsing bacterial vaginosis (gardnerellosis) was conducted by the Pavlodar City Centre for Clinical Immunology and Reproduction in 2000. This vaginal infection is prone to relapses in more than 30% of treated cases. Twenty two women completed treatment for two months without any relapses<sup>28</sup>.

In 1995 a clinical study by NZ naturopaths was conducted on the efficacy of Kolorex Intimate Care cream on twenty-six women suffering from vaginal candidiasis. Twenty-three of the women (89%) reported relief when the cream was applied 2 or 3 times daily for a week. Of the remaining three women, two reported a gradual increase in symptoms and 1 found the cream too hot to use a second time<sup>29</sup>.

## Scientific Summary

### Kolorex™ Products

The leading products that Forest Herbs Research Ltd has developed from RedLeaf Horopito are:



Kolorex Advanced Candia/Candida Care soft gels for intestinal dysfunction and Candidiasis



Kolorex Foot and Toe Care Cream for athlete's foot and nail fungi



Kolorex Intimate Care cream for intimate itch and vaginal thrush



Kolorex Mouth and Throat Care tea for oral thrush and hygiene

## Scientific Summary

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