

Kolorex[®] Horopito – Scientific Summary

TABLE OF CONTENTS

Horopito – the ancient herb	2
Traditional Use	2
Anti- <i>Candida</i> Discovery	2
Kolorex [®] 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	8
Stability studies	9
Clinical Studies	10
References	11



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 the 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 ⁽¹⁾.

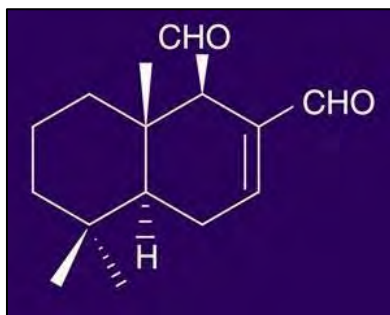
Traditional Use

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 and were rubbed on the breasts for weaning infants ⁽²⁾. 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' ⁽³⁾.

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 1 whereas the inhibitory effect of Amphotericin B against *C. albicans* required 3–4 days incubation to become effective ⁽⁴⁾. See Table 1.

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

Concentration (ug/disc)	Time (days)				
	1	2	4	8	11
Polygodial					
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
Amphotericin 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

Kolorex® Horopito

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



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 ⁽⁶⁾. Kolorex 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 ⁽⁷⁾.

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 ⁽⁸⁾. 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 ⁽⁸⁾. EDTA is another food preservative upon which polygodial exerts a synergistic effect, presumably by facilitating its transport into

yeast cells ⁽⁹⁾. *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 ⁽¹⁰⁾.

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 ⁽¹¹⁾.

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 ⁽¹¹⁾.

Toxicology and Mutagenicity

Toxicological assessment of Kolorex Horopito and 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 ⁽¹²⁾.

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 ⁽¹²⁾.

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 1 million capsules containing milled Kolorex Horopito have been sold annually per year for the last 13 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 1 million soft gel capsules containing Kolorex 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. Two have been severe and relate to allergic reactions. In response to this Forest Herbs Research is introducing instructions to skin test the cream for any allergic reaction before using on intimate areas. No adverse reactions have been reported for Kolorex Foot and Toe Cream

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

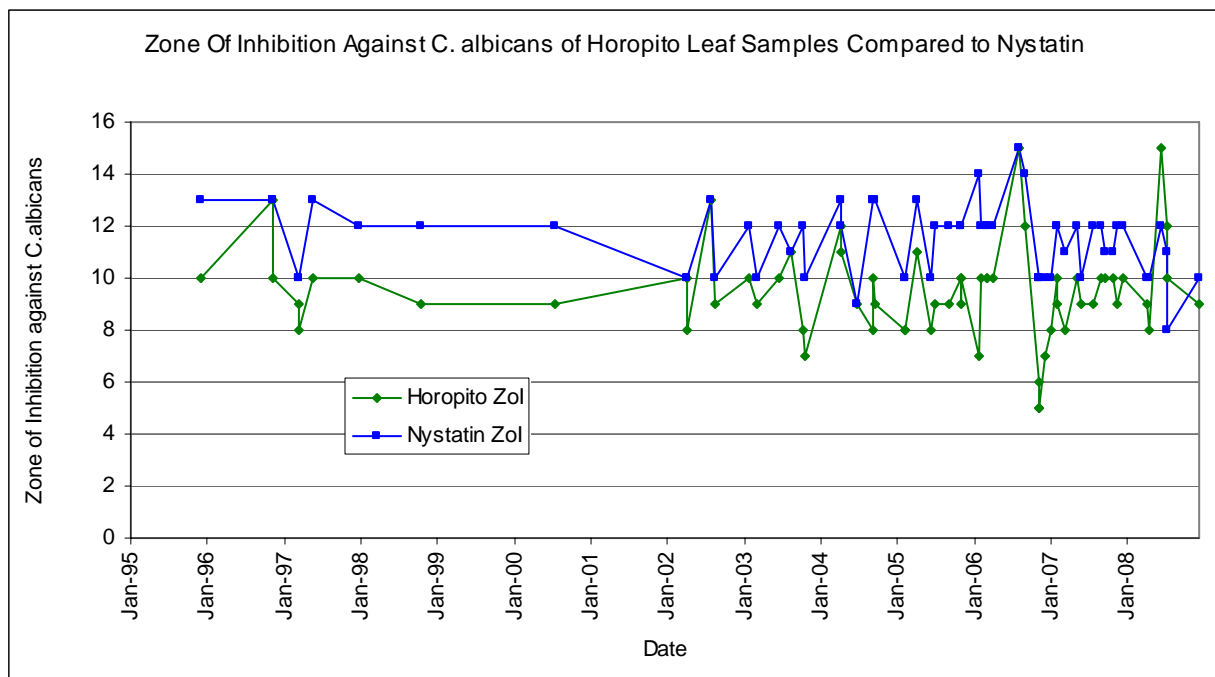
Each batch of Kolorex Horopito leaves is independently assayed and must reach a specified level of anti *Candida* activity (against a pharmaceutical control) before it is used in Kolorex products.

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.

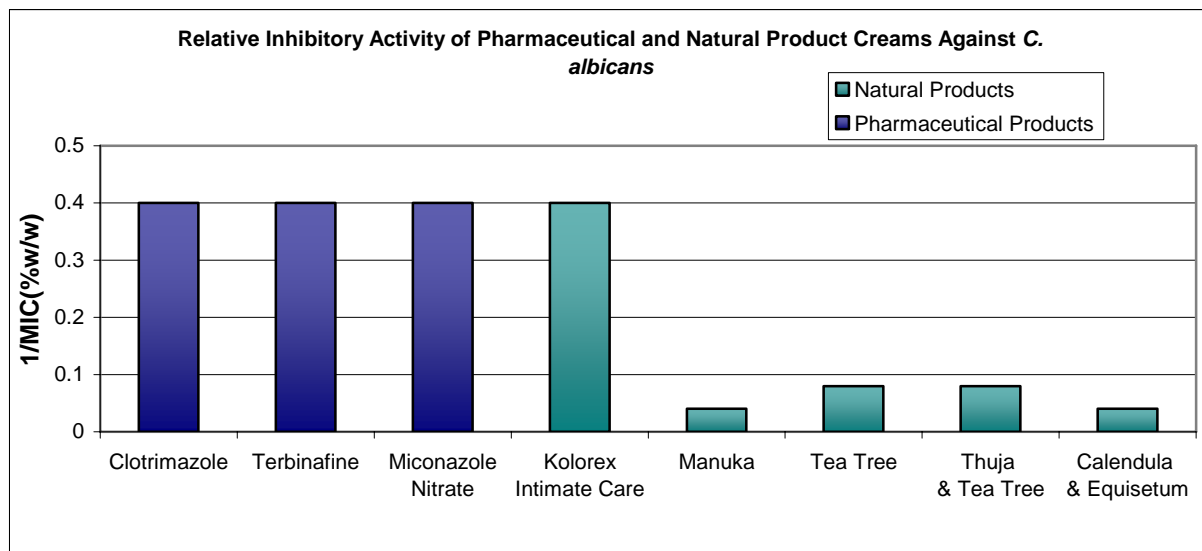
Figure 3. Horopito Leaf Zone of Inhibition (Zoi) Test Results ⁽¹³⁾



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 4).

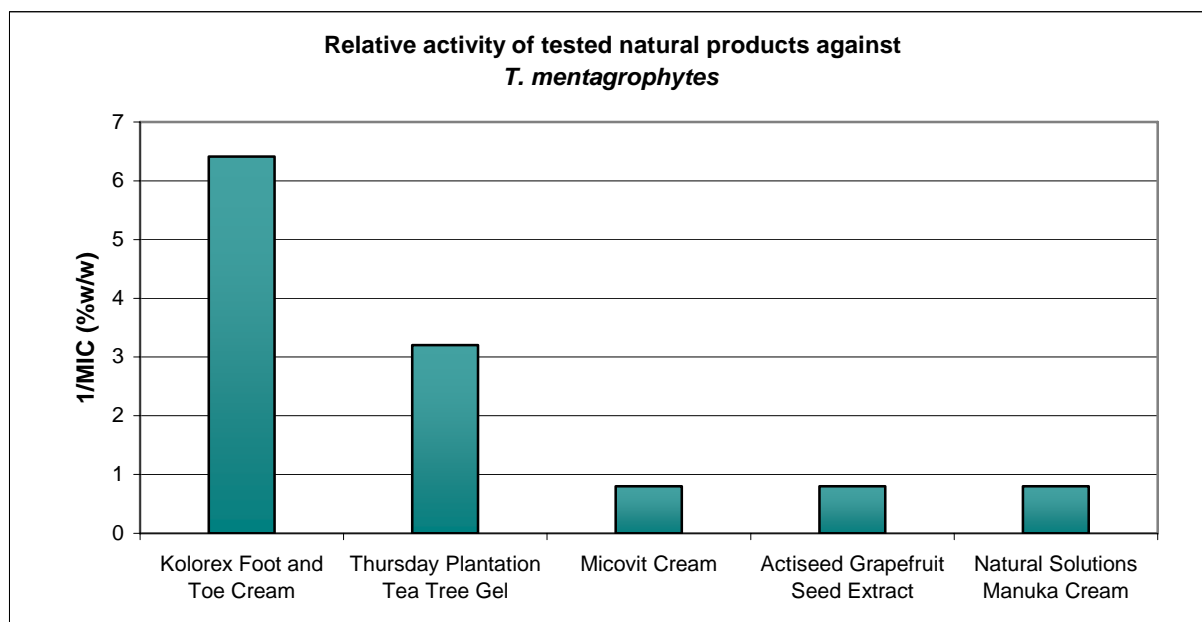
Figure 4. Kolorex Intimate Care Cream inhibitory activity compared with other products ⁽¹⁴⁾.



Kolorex Foot and Toe Cream

The testing of Kolorex Foot and Toe cream (See Figure 5 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 ⁽¹⁵⁾.

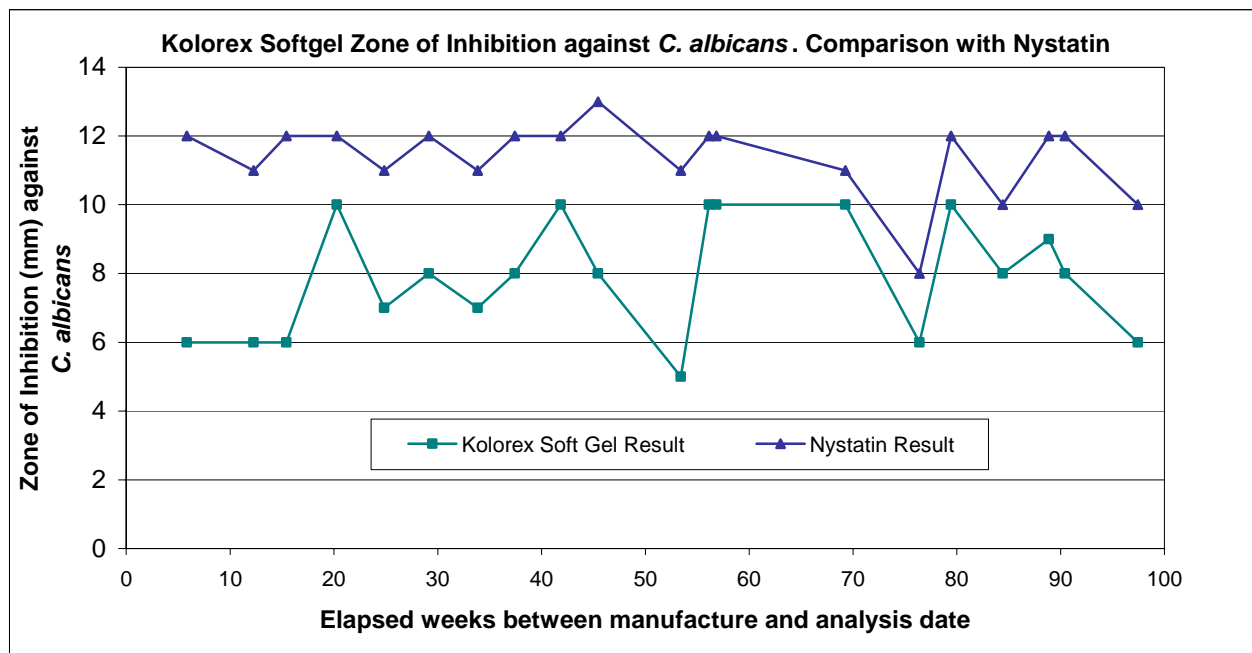
Figure 5. Relative activity against *T. mentagrophytes* of Kolorex Foot and Toe Cream compared to other natural products tested.



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 antiyeast product Nystatin as a control. See Figure 6. After 94 weeks the softgels are inhibiting the growth of *C. albicans* as effectively as when they were manufactured ⁽¹⁶⁾.

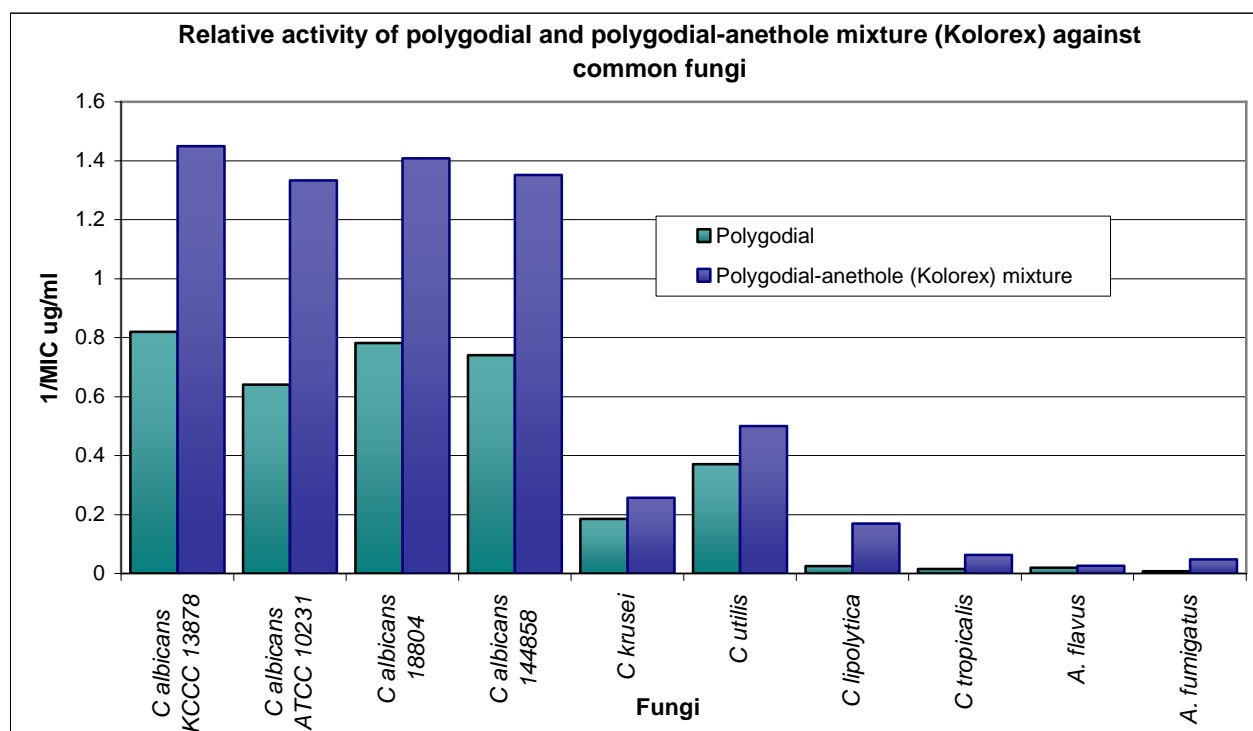
Figure 6. Kolorex Soft Gel Zone of Inhibition against *C. albicans* test results compared with Nystatin



Kolorex Horopito and Aniseed Capsules

Kolorex Horopito and Aniseed capsules exhibit a wide profile of antifungal activity of in terms of both effectiveness and spectrum of action. This discovery by a group of researchers led by Metugriachuk ⁽¹⁷⁾ found that the remarkable in vitro antifungal activity of the Kolorex mixture was better than polygodial alone (as illustrated in Figure 7). Furthermore they found that there was no significant toxicity associated with Kolorex. The most commonly used antibiotics however have the disadvantage of toxic side-effects ⁽¹⁷⁾. Their study concluded that “Kolorex is ... expected to be a promising compound for the development of therapeutic regimens acting through a synergistic effect”.

Figure 7. Relative activity of polygodial and polygodial-anethole mixture against common fungi⁽¹⁷⁾



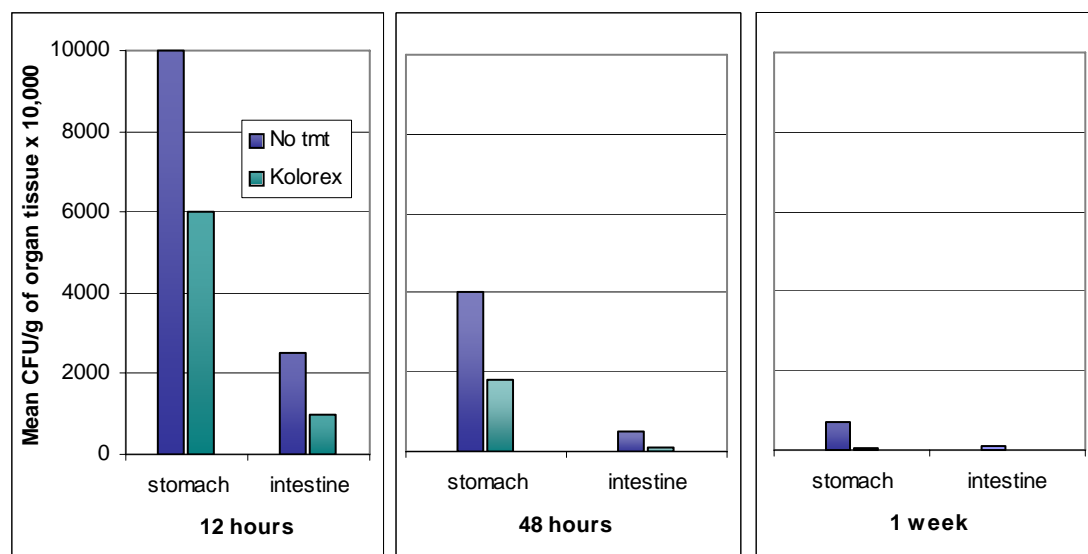
Kolorex Mouth and Throat Tea

Results from research by Nakajima and colleagues⁽¹⁸⁾ 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⁽¹⁹⁾ 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 8). 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 8. *C. albicans* concentration in tissue tested: Effect of 48hr pre-treatment with Kolorex (19).



Further research by Marotta and associates (20) 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 (18) 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 and 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.

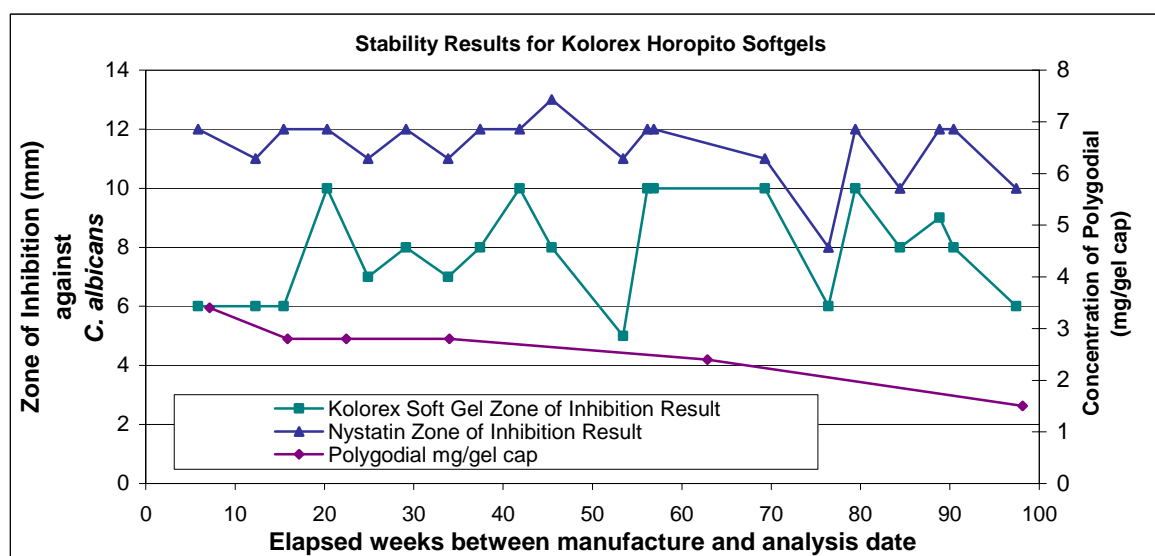
Stability studies

Kolorex Horopito softgels

The softgels are as effective *in vitro* against *Candida albicans* at 94 weeks, as they were immediately after manufacture (21) (see zone of inhibition results Figure 9).

The stability of the active ingredient, polygodial, is being studied under controlled storage conditions by Southern Cross University, Australia. These results are also graphed in Figure 9.

Figure 9. Kolorex Advanced Intestinal Care 500mg Soft Gel Capsule Stability Over 100weeks. Zone of Inhibition Results against *C. albicans* compared with Nystatin and Concentration of Polygodial (mg/gel cap)



Clinical Studies

Kolorex Capsules

An open study conducted by eleven NZ naturopaths in 1992 for Forest Herbs Research examined the therapeutic effect of capsules containing milled Horopito and milled Anise seed in patients diagnosed with chronic intestinal candidiasis. This study demonstrated a symptom improvement rate in 76% of cases ⁽²²⁾.

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 Horopito and aniseed capsules with 10 patients administered fluconazole (Diflucan, Pfizer). All patients taking fluconazole and 90% of patients taking Horopito and aniseed capsules showed a significant improvement after 7 and 14 days respectively ⁽²³⁾.

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 ⁽²⁴⁾.

In 1995 a clinical study by NZ naturopaths was conducted on the efficacy of Kolorex Intimate Care Cream on 26 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 3 women, 2 reported a gradual increase in symptoms and 1 found the cream too hot to use a second time ⁽²⁵⁾.

References

1. Webb C, Johnson P, and Sykes B. *Flowering Plants of NZ*, DSIR, 1990, p104
2. Brooker S G, Cambie R C, and Cooper R C. *New Zealand Medicinal Plants*, Heinemann, 1987, p240
3. Riley M. *Maori Healing And Herbal*, Viking Sevenses, 1994, pp146–148
4. MaCallion R F, Cole A L J, Walker J R L, Blunt J W, Monro M H G. *Antibiotic Substances from New Zealand Plants*, *Planta Medica*, 1982, vol 44, pp134–138
5. Larsen L. Crop & Food Research Ltd, On FHR file
6. Mylek M. *Development and Analysis of Extracts of Polygodial from Horopito*, 1999, Industrial Research Ltd, Report No.904
7. Lee S H, Lee J R, Lunde C S, Kubo I. *In Vitro Antifungal Susceptibilities of Candida albicans and other Fungal Pathogens to Polygodial, a Sesquiterpene Dialdehyde*, *Planta Medica*, 1999, vol 65, pp205–208
8. Kubo I and Lee S H. *Potential of Antifungal Activity of Sorbic Acid*, *J. Agric. Food Chem.*, 1998, vol 46, pp4052–4055
9. Kubo I, Lee S H, Ha T J. *Effect of EDTA Alone and in Combination with Polygodial on the Growth of Saccharomyces cerevisiae*, *J. Agric. Food Chem.*, 2005, v53, No 5, pp1818–1822.
10. Fujita K and Kubo I. *Naturally Occurring Antifungal Agents against Zygosaccharomyces bailii and Their Synergism*, *J. Agric. Food Chem.*, 2005, 53, pp 5187–5191
11. Kubo , Fujita K, Lee S H, Ha T J. *Antibacterial Activity of Polygodial*, *Phytotherapy Research*, 2005, 19, pp 1013–1017
12. Eric Winkelman. *Toxicological Assessment of Horopito (Pseudowintera colorata)*, 2008. GlaxoSmithKline Memorandum
13. Forest Herbs Research. Horopito Leaf Zone of Inhibition against C. Albicans Data on File
14. Forest Herbs Research. Kolorex Intimate Care Cream MIC Data on File
15. Forest Herbs Research. Kolorex Foot and Toe Cream MIC Data on File
16. Forest Herbs Research. Kolorex Horopito Softgel Zone of Inhibition Data on File
17. Metugriachuk Y, Kuroi O, Pavasuthipaisit K, Tsuchiya J, Minelli E, Okura R, Fesce E, Marotta F. *In View of an Optimal Gut Antifungal Therapeutic Strategy: and in vitro Susceptibility and Toxicity Study Testing a Novel Phyto-compound*. *Chinese Journal of Digestive Diseases*, 2005, vol 6, pp98–103.
18. Nakajima J, Papaah P, Yoshizawa M, Marotta F, Nakajima T, Mihara S, Minelli E. *Effect of a novel phyto-compound on mucosal candidiasis: Further evidence from an ex vivo study*. *Journal of Digestive Diseases*, 2007, vol 8, pp48–51
19. Naito Y, Wu C C, Seal M G, Gelosa F, Yoshioka M, Safran P, Marotta F. *Protective Effect of a Polygodial/Anethole-Containing Natural Product against Candida albicans Gastrointestinal Colonization and Dissemination*. *International Medical Journal*, 2001, vol 8, No 1, pp3–9.
20. Marotta F, Barreto R, Kawakita S, Minelli E, Pavasuthipaisit K, Lorenzetti A, Nishiwaki M, Gelosa F, Fesce E, Okura R. *Preventative strategy for Candida gut translocation during ischemia-reperfusion injury supervening on protein-calorie malnutrition*. *Chinese Journal of Digestive Diseases*, 2006, v7, pp33–38.
21. Forest Herbs Research. Soft Gel Stability Trial Data on File
22. 1992 NZ Naturopath Study, 1992. Data on File, Forest Herbs Research Limited, Nelson, New Zealand.
23. 1997 Pavlodar study. Pavlodar City Centre for Clinical Immunology and Reproduction. Head Physician: O. Ogorodnikova. Pavlodar. Data on File, Postgraduate Physicians' Training Faculty Assistant: M. Valivach.
24. Forest Herbs Research. Kolorex Cream 2000 Pavlodar Clinical Study Data on File
25. Forest Herbs Research. Kolorex Cream 1995 Clinical Study Data on File. Training Faculty Assistant: M. Valivach.