"NATURAL PRESERVATIVES"
Anthony C. Dweck

Research Director, Peter Black Medicare Ltd., White Horse Business Park, Aintree Avenue, Trowbridge, Wiltshire, UK. BA14 0XB

SUMMARY

This paper looks at the theoretical development of a natural preservative system using the author's database on medicinal plants as a source of references. The legal aspects of this concept are considered.

The traditional methods of preservation, many taken from the food industry are summarised. The use of alcohol, glycerine, sugar, salt, dessication, anhydrous systems and temperature are amongst examples considered.

The definitions of the many words used to describe the act of preservation are considered, and the confusion that results from the presence of the many synonyms is considered. e.g. antimicrobial, antibiotic, antiseptic, bactericidal, etc.

Specific organisms are identified as being of particular interest, especially those standard organisms that form part of the B.P. challenge test. These include Candida albicans, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger and Staphylococcus aureus. A cross-section of plants mentioned in the literature as being specifically targeted at these organisms are considered.

The paper concludes with Appendices of plant materials that have mention in the literature according to specific definitions, which may give researchers a potential introduction to future research.

KEY WORDS

Natural preservation, traditional preservation, challenge test organisms, legal status.

INTRODUCTION

The subject of natural preservatives is one that probably has more academic interest than practical or economic virtue. However, it does have a wonderful marketing angle which may justify the higher raw material costs.

The paper first reviews the most commonly used methods of preservation that are already available to the formulator. The food and beverage industry may be called upon for many of these examples.

Secondly, the paper moves on to consider the search through the existing data and considers the problems of commonly used synonyms for the act of preservation.
Finally, the author looks at some specific organisms commonly encountered in the cosmetic and toiletry industry and gives examples of some of the plant references.

**LEGAL POSITION**

No preservative may be used which does not appear in:


However, there is no legislation for those natural materials, which, when used for their beneficial effect on the skin, may coincidentally have a positive effect on the total preservative requirement of the formulation. Of course, no material appearing in Annex II may be considered.

**SUGAR**

High levels of sugar can preserve against spoilage organisms, this may be seen in jams, preserves, certain sweet pickles and marmalades. This is also an important factor in the preservation of boiled sweets and chocolates etc. Increasingly, it will be noticed that many products now have to be kept in the refrigerator or freezer once opened, because sugar has been replaced by artificial sweetener which is cheaper and healthier(?) to eat, but which compromises the self-preservation of the product.

**HONEY**

Honey in its undiluted form is also a natural preservative and, indeed, there are many learned papers citing honey as a viscous barrier to bacteria and infection.

**ALCOHOL**

Not all organisms are bad! The production of alcohol from sugar by yeast is an industry in its own right. A wine carefully produced using sterilised equipment and fermented to 13% by volume will just about resist further infection from external organisms, once the ferment has completed. It is during the time of the fermentation process that the fermenting must is vulnerable to infection. The naturally produced fermentation grade alcohol can be concentrated by distillation and used as a natural preservative in toners, aftershaves and colognes.

**HEAT**

Heating, cooking and pasteurisation is another natural form of preservation that will sterilise products, especially where that product is designed as a one-shot use product -
for example, a phial or a sachet. Alternatively, once opened, the product can be stored in the fridge or freezer to prevent microbiological degradation.

**DESICCATION**

Removing water from a product or making it totally dehydrated will greatly reduce the possibility of spoilage; however, it must be recognised that the presence of spore-bearing organisms could become active once that water is reintroduced.

**ANHYDROUS**

In a similar vein, one could make products with materials that do not contain any traces of water, i.e. to deliberately design and formulate a totally anhydrous product. However, creams that can be finished by the consumer, by introducing water to the blend of oils, fats and waxes are prone to the same restrictions as the dessicated products.

**SALT**

The use of extreme levels of salt as used by the ancient mariners to preserve their meat is effective and it very likely that the preservation of the Egyptian mummies was, in part, achieved by the 40 day treatment in natron (a concentrated brine solution that osmotically drained the tissues of water).

**COLD**

Placing a product in the cold merely 'stops the clock' on microbiological growth and this is perfectly fine, provided the product was sterile when it was placed in the cold and/or had sufficient preservative 'mass' to counter any new organisms subsequently introduced.

**ACID pH**

The preservative activity can be boosted by operating at as low a pH as possible. Natural acidity could be obtained from one of the many of the alpha hydroxy acids (AHAs) which are obtained from citrus species, where the major components are citric and malic acids.

Incidentally, it is surprising that expensive sources of natural alpha hydroxy acids are being contrived, when the producers of baobab oil are throwing away large quantities of tartaric acid as a part of their waste product.

**CHELATING AGENTS**
In addition to formulating at low pH, chelating agents such as ferulic acid extracted from rice bran, could be added to enhance the activity of the natural preservative.

ANTIOXIDANTS

Antioxidants such as natural tocopherol and ascorbic acid will further aid in preservation, as well as reducing the potential rancidity.

GLYCERINE

High levels of vegetable glycerine, up to 15-20%, will also have a preservative effect, similar to that effect obtained by the use of high levels of sugar.

PLANTS SELF-PRESERVATION

Plants in the wild do not go mouldy, and yet they are in an environment that predisposes them to suffer from the infestation of all manner of spoilage organisms. Yeasts, moulds and bacteria abound in the soil, all working to breakdown dead plant material and provide fresh humus for those plants living in the soil. Living plants resist the natural forces of disintegration.

The chemicals present in all parts of the plant protect it from the environment. However, examples can be seen where tampering with the plant leads to a reduction in the efficacy of this natural mechanism.

Consider the rose. A highly refined cultivar rose, which has been selectively bred for its flowers, loses much of its immunity and is prone to black spot, mould and mildew. The older, original rose stock (*Rosa sinensis*) from which the cultivar has been partially developed, remains unaltered, unbred and totally oblivious to the blights and blemishes of its modified offspring.

It is concluded, that the chemical constituents within each plant clearly differ in composition, even though the older rose is a direct genetic relative of the cultivar. Furthermore, that there is a chemical or group of chemicals present in the plant that is capable of killing micro-organisms. This chemical composition varies according to whether the plant is alive or dead, and in certain/most plants will vary according to season.

In many cases, when these plants are extracted, it is found that the extracts are capable not only of resisting certain spoilage organisms, but in some cases can actively act to destroy them.

It is this phenomenon that is of interest to us.

NATURAL PRESERVATIVES AND DEFINITIONS
It was realised that the possibility of using plants as natural preservatives was achievable. The data base was quizzed for those plants that were capable of killing microorganisms.

There are numerous words to describe the "killing of micro-organisms", these are listed, together with their definitions in Appendix I, namely, words such as antibiotic, antibacterial, bactericide, etc.

Computers are not very good at handling synonyms, and searching data using up to ten synonyms simultaneously results in a botanical list that is composed of hundreds of materials. In order to simplify the task, the searches were carried out on specific words in order to isolate groups of active plants relevant to specific definitions. Please see Appendices III - IX (smaller files have been omitted to save space!)

It is obvious that for a fast result, one needs to be more specific and more selective. These huge lists of possibles are fine for the researcher with lots of time, but not very helpful for the chemist needing a quick result.

SPECIFIC ORGANISMS

The safest way to look for plant preservation is to search for activity against classes of organisms, for example at yeasts, moulds and bacteria (Gram +ve and Gram -ve) as in Appendix II.

This approach was far more specific than the search against general words, and we extended the search into specific organisms.

The following organisms were considered appropriate:-

I. Candida albicans
II. Pseudomonas aeruginosa
III. Escherichia coli
IV. Aspergillus niger
V. Staphylococcus aureus

Obviously at this very specific level of enquiry, the amount of data is considerably reduced. A quick examination of the data revealed the following typical references for each of the organism searched.

I CANDIDA ALBICANS

A cross section of typical references for a few natural materials, relevant to Candida albicans.

Calamintha officinalis
Tony Balacs\textsuperscript{1} reported that Savory, calamintha and thyme were all very active in vitro against \textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, \textit{Saccharomyces cerevisiae} and \textit{Candida albicans}.

\textit{Cryptolepis sanguinolenta} Schltr.
\textit{Cryptolepis obtusa} N.E.Brown

Alexandra Paulo, Aida Duarte, Peter Houghton and Elsa Gomes\textsuperscript{2} reported that species of Cryptolepis are used in traditional African medicine for a variety of purposes. The roots and leaves decoction of \textit{Cryptolepis obtusa} N.E.Brown is used in Mozambique mainly as an anti-abortive and antiparasite. The roots and leaves of this species purchased in Maputo, were screened for their antimicrobial activity and chemical content.

The MIC of the ethanolic and petroleum ether extracts were determined amongst many organisms including \textit{Candida albicans} CIP3153A by the twofold serial broth microdilution assay in concentration ranging from 5 mg/ml to 100 $\mu$g/ml.

\textit{Satureia hortensis}
\textit{Satureia montana}

Valnet\textsuperscript{3} says that \textit{Satureia montana} - Winter Savory, enjoyed great prestige in antiquity as a digestive and in certain healing remedies. It contains pinene, carvacrol 30-40\%, cymene 20-25\%, terpenes 40-50\%, cineol, and a small amount of thymol. The Pharmacological Faculty at Montpelier did a study on the antibacterial and antifungal properties. 10 types of \textit{Staphylococcus}, 14 other microorganisms and 11 fungi were examined including \textit{Candida albicans}, \textit{C. tropicalis}, \textit{Trichophyton interdigitalis}. The results were very encouraging. In this respect it was equal to thyme in performance.

![Carvacrol](image)

Tony Balacs\textsuperscript{1} says that savory oil was rich in carvacrol (56.8\%), and that it was very active in vitro against \textit{Candida albicans}.

\textit{Litsea cubeba}

Tony Balacs\textsuperscript{4}, says that \textit{Litsea} is used as a commercial source of citral. Citral accounts for 75\% of May Chang oil and has two isomers which are neral and geranial, which are the respective aldehyde equivalents of nerol and geraniol. May chang oil contains slightly more geranial (41\%) than neral (34\%). Citral is known to be antitumoral and antifungal and to help prevent experimental atherosclerosis. The antitumoral evidence is based on the clinical use of citral (and citronellal) in Japan during the 1940s.
on 125 people with cancer. In six (5%) cases a ten-year follow up showed complete cure. Full report IJA Vol.1, No.4 / Vol.2, No.1.

The antifungal effects of citral and lemongrass oil were published in IJA Vol.3 No.1. Citral showed significant action against *Microsporum gypseum*, *Aspergillus fumigatus*, *Trichophyton mentagrophytes* var. *interdigitale* and *Candida albicans*.

*Plumbago zeylanica*

Greenburg⁵ reports that the root has been shown to contain plumbagin, a yellow naphthoquinone, which is responsible for its antimicrobial and antibiotic activity. (Bep Oliver-Bever²⁵, N.Atkinson and H.E.Brice³²).

A very dilute solution (i.e. a concentration of 1:50,000) of plumbagin is lethal to a wide spectrum of bacteria and to pathogenic fungi, i.e. *Coccidioides imminites*, *Histoplasma capsulatum*, *Trichophyton* spp., *Candida albicans*, *Aspergillus niger* and *A. flavus*. (F.A.Skinner³³).

*Lapacho colorado*  
*Lapacho morado*

In the Lawrence review of Natural Products⁶ (July 1990) on Taheebo.

According to reports in the Brazilian and American lay press, teas prepared from the inner bark of these trees have been used for centuries to treat various diseases. Extracts of the plant have recently been used topically for the management of *Candida albicans* infections.

Chemical analysis of taheebo has lead to the isolation of numerous quinone constituents and a variety of minor compounds from the inner bark and heartwood. These
include the naphthaquinones lapachol and β-lapachone, and the anthraquinone tabebuin. Lapachol is present at a level from 2% to 7%.

Lapachol and the related compound xyloidone have been assessed for antimicrobial activity; lapachol was active against gram positive and acidfast bacilli, but inactive towards yeast and fungi, while xyloidone was active against *Brucella* and *Candida*. Lapachol is an active antimalarial and antitrypanosome. Aqueous extracts of Taheebo have been shown to be inactive against *Candida* cultures.

*Melaleuca alternifolia*

Vicki Home\(^7\) discusses the level of cineole found in tea tree oil. She has been working on a range of products designed for use on the vaginal area and has been looking at the optimum composition of tea tree oil for the treatment of *Candida albicans*.

There is a general decline in activity against candida as the levels of the following components decreases: alpha-terpinene, gamma-terpinene, terpinolene, terpinen-4-ol, and as the following compounds increase: cineole, limonene, alpha-terpineol.

The Lawrence review\(^6\) of Natural products (Jan 1991) on Tea Tree Oil, the oil was found to have an MIC of 0.5% v/v for *Aspergillus niger* and *Trichophyton*, and 0.025% v/v for *Candida albicans*.

Price\(^8\) says it is also used for colds, mouthwash and sore throat, for bronchitis, candida, infected wounds and insect bites.

Rosalind Blackwell\(^9\) reports that tea tree oil which has optimal activity against *Candida albicans* is not the one with optimal activity against moulds and yeasts usually prefer an acidic medium and grow most rapidly at temperatures of 22-25 C, whereas gram positive and gram negative bacteria prefer an alkaline medium and warmer temperatures.

The terpenes in tea tree mix with the sebaceous secretions in such a manner as to penetrate the top layers. They thus carry the disinfection properties deeper than many emollient creams.

It was also used to combat infections in the gut, e.g. *Candida albicans*. It contains a variety of terpenes, which are insoluble in water. The terpene paracymene has an analgesic action on the skin. Terpenes stimulate the adrenal cortex; they are anti-viral but also immunomodulant, influencing the immunoglobulins and counteracting the inflammatory reaction.
Home, V.N., Williams, L.R., Asre, Saras\textsuperscript{10} report that the antimicrobial activity was discovered in 1920's when Penfold and Grant reported that the essential oil extracted from \textit{Melaleuca alternifolia} was 11 times more potent than phenol, which at that time was one of the most potent antiseptics in commercial use.

The Rideal Walker phenol coefficient provided an instant means of quantifying the antiseptic properties with phenol rated as 1. The oil was not only more potent than phenol, but it was also not as irritant to skin and open wounds. Phenol is in fact a very caustic material and causes irritations and burns to the skin.

The paper shows some comparative Rideal Walker values. The major component of the oil is terpinen-4-ol which has a value of 16.0, the chloroxylenol in Dettol has a value 60.0. Tea tree oil by modern standards is not a powerful antiseptic agent.

The Rideal Walker test has been superceded by the Kelsey-Sykes test which forms the basis of the Therapeutic Goods Act (TGA) test for antiseptics and disinfectants for hospital use, having the advantage that it simulated the conditions under which disinfectants are normally used. The test is designed for water soluble materials, and so tea tree oil is again at a disadvantage.

The authors went on to discuss comparative evaluations that had been done with other natural oils, especially zones of inhibition against \textit{Candida albicans} (the yeast which causes thrush). With thyme there was no growth, cinnamon 18mm, terpinen-4-ol 6mm, bergamot no zone, sandalwood no zone.

\textit{Melaleuca alternifolia} contains 1,8-Cineole at around 4% and terpinen-4-ol is present at greater than 35%. The concentration of oil used against \textit{Candida albicans} was 0.5%.

Tea tree oil passed the USP (XXII) and the British Pharmacopoeia challenge test against the above at 0.8% v/v.

Manufacturing Chemist\textsuperscript{11}. The production of Australian Tea Tree oil now surpasses 100 tpa. The oil has been shown to have antimicrobial activity, varies with micro-organisms.

The anti-microbial activity of the oil correlated well with the terpinen-4-ol level of the oil for \textit{Candida albicans}. However, there was no simple correlation between terpinene-4-ol levels of the oils and their activity against \textit{Staphylococcus}, suggesting that for this particular micro-organism, some other components of the oil were responsible for a significant proportion of the overall antimicrobial activity.

For \textit{Candida albicans}, the activity of terpinen-4-ol was much greater than that of the standard oil, indicating that the 35-40% of terpinen-4-ol in the oil is the major contributor to its antimicrobial activity and suggesting that oils with higher terpinen-4-ol levels should be more active. For \textit{Staphylococcus} the terpinene-4-ol. For \textit{Candida albicans} p-cymene was more active than the standard oil, but not as active as terpinen-4-ol. Although p-cymene is usually only present at 2-5% in commercial tea tree oil, its
powerful antimicrobial activity makes a significant contribution to the oil's overall activity.

*Melaleuca leucadendron*

Tony Balacs\(^\text{12}\) reports that 1,8-cineole, (-)-linalool, (-)-terpinen-4-ol and \(\alpha\)-terpineol were all very active against *E. coli* in vitro, less so, but still markedly active against *S. aureus*, (-)-linalool was the most active constituent, whereas against *P. aeruginosa*, terpinen-4-ol and \(\alpha\)-terpineol came out top. Several *Streptococcus* species, and the fungus *Candida albicans*, were all found to be sensitive to all four constituents, but *Enterobacter* was only sensitive to 1,8-cineole.

*Cinnamomum zeylanicum*

Leung\(^\text{13}\) says that Cinnamon oil has antifungal, antiviral, bactericidal and larvicidal properties. A liquid carbon dioxide extraction at 0.1% has been demonstrated to suppress the growth of many organisms, including *E. coli*, *Staphylococcus aureus*, and *Candida albicans*.

*Usnea barbarta*

In a paper by James and Mitchell\(^\text{14}\) presented at a symposium in London in we read that Alpine Lichen is cited as a source of Usnic acid, which was first extracted in 1843 by Rochleder and Heldt, and it is chemically dibenzofuran or 6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3-(2H,9bH)-dibenzofuran-1-one.

The paper listed the minimum inhibitory concentration for *Candida albicans* (ATCC 10231) as 25-74 \(\mu\)g/L.

*Helichrysum angustifolium DC.*

Leung\(^\text{13}\) refers to *Helichrysum angustifolium* DC. [Syn. *H. italicum* G.Don; *H. italicum* (Roth) Guss.] Fam. Compositae or Asteraceae. Known as Immortelle, Helichrysum or Everlasting.

The volatile oil of *H. italicum* flowers has been reported to exhibit antimicrobial properties in vitro against *Staphylococcus aureus, Escherichia coli*, a *Myobacterium* species, and *Candida albicans*. High activities were observed in oil samples containing higher concentrations of nerol, geraniol, eugenol, \(\beta\)-pinene, and furfurol.

*Pinus silvestris*
Research Reports. The young oil was inactive against Gram-negative organisms such as E.Coli, but active against the Gram-positive Staph.aureus, Strep.faecalis, Bacillus subtilis, other Gram-positive organisms and against the yeast, Candida albicans (commercial oil was ineffective against this organism). The spectrum of activity of commercial oil was broadly similar but this oil was consistently weaker in effect than the young oil.

In a useful book by Jeffrey B. Harborne and Herbert Baxter we read of a number of sesquiterpene lactones that have good effect against Candida.

Dihydromikanolide, which occurs in climbing Hempweed, Mikania scandens, and many other Mikania spp. Glaucolide B which is found in New York ironweed, Vernonia glauca (= V. noveboracensis) and many other Vernonia spp. (Compositae). Mikanolide, found in the climbing hempweed, Mikania scandens, and in M. batatifolia, M. cordata, M. micrantha and M. monagasensis (Compositae). Pseudoivalin, which occurs in Iva microcephala and Calocephalus brownii (Compositae).

Cymbopogon citratus
Adropogon citratus

In the International Journal of Aromatherapy (Vol.3 No.1) we read that the antifungal activity of lemongrass oil has been evaluated using fungistatic (MIC and agar diffusion tests) and fungicidal (spore germination) studies. Appreciable activity was observed against various isolates of Candida and clinical isolates of Aspergillus fumigatus, Microsporum gypseum and Trichophyton mentagrophytes. The most resistant organism was A. fumigatus while M. gypseum and the Candida spp were the most susceptible of the isolates. Comparative studies with pure samples of citral and citronellal, constituents of lemongrass oil, showed good activity against the test fungi while dipentene and myrcene showed no activity.

Eucalyptus globulus

Of the oils tested, E. citriodora was the most effective inhibitor especially of Candida albicans.

Passiflora incarnata

The Lawrence review of natural products (May 1989)

In vitro experiments have demonstrated that passicol kills a wide variety of molds (moulds), yeasts, and bacteria. Group A haemolytic streptococci are much more susceptible than Staphylococcus aureus, with Candida albicans being intermediate in susceptibility. The antimicrobial activity of passicol disappears rapidly from dried plant residues and fades gradually in aqueous extracts. Addition of dextran, milk, or milk products has a stabilising effect on dry passicol.

Allium sativum
Trattler\textsuperscript{17} lists numerous benefits of garlic, including yeast infections (\textit{Candida albicans}) of the skin or mucous membranes.

In a technical data sheet from Alban Muller we read that the bulb contains 0.1-0.4\% of a volatile oil composed of alliin or S-methyl L-cystein sulphotoxide. Allicin is the major odour principle that is produced by the enzymatic action of alliinase on alliin. The bulb contains as well about 17\% of proteins, mineral matters and vitamins (B1, B2, PP, C). However, the main components of garlic are fructosans which account for up to 75\% of the dried weight.

The smell and the bacteriostatic and antifungal properties are due to the sulphur containing compounds. They are particularly efficient against dermatophytis and pathogenic yeasts (\textit{Candida}).

\textit{Echinacea angustifolia}

Glenise McLaughlin\textsuperscript{18} gives a long list of indications which includes the treatment of \textit{Candida} infections.

Macrophages from different organs could be activated to produce interleukins 1 and 6 and tumour necrosis factor, to produce elevated amounts of reactive oxygen intermediates and to inhibit growth of \textit{Candida albicans} in vitro. In vivo the polysaccharides could increase proliferation of phagocytes in the spleen and bone marrow and the mitigation of granulocytes to the peripheral blood.

She cited that recently recurrent vaginal candida infections were treated with \textit{Echinacea} cream and liquid extract and a cream alone. The extract was more effective than the cream alone.
Propolis

Allan Onions\textsuperscript{19} talking of propolis. Early work on propolis suggested widespread antibacterial activity, but more recent studies have confirmed that the activity is restricted to certain bacteria, with good results having been recorded against \textit{B. mesentericus}, \textit{M. lysodeicticus}, \textit{P. vulgaris}, \textit{S. aurens} and \textit{Strept. cremoris}. Propolis does, however, show excellent antifungal activity, particularly against \textit{Candida albicans}, \textit{E. inguinalis}, \textit{E. rubrum} etc.

II PSEUDOMONAS AERUGINOSA

\textit{Calamintha nepeta}

1. Tony Balacs\textsuperscript{1} reports that an Italian group analysed the essential oils of four Mediterranean Lamiaceae for antimicrobial activity. \textit{Satureia montana}, \textit{Thymus vulgaris}, \textit{Calamintha nepeta} and \textit{Rosmarinus officinalis} oils were all found to be active.

The calamintha oil was rich in pulegone (46\%) and para-cymene (17\%).

\begin{center}
\includegraphics[width=0.5\textwidth]{pulegone.png}
\end{center}

Calamintha was moderately active against \textit{Pseudomonas aeruginosa}.

The authors suggested that the antimicrobial activity of the oils resided in their respective contents of thymol, carvacrol, pulegone, menthone, terpinene and cymene.

\textit{Cryptolepis obtusa} N.E.Brown

Houghton and Gomes et al.\textsuperscript{2} report that species of \textit{Cryptolepis} are used in traditional African medicine for a variety of purposes. The roots and leaves decoction of \textit{Cryptolepis obtusa} N.E.Brown is used in Mozambique mainly as an anti-abortive and antiparasite. The roots and leaves of this species purchased in Maputo, were screened for their antimicrobial activity and chemical content.

The MIC of the ethanolic and petroleum ether extracts were determined for \textit{Escherichia coli} ATCC 25922, \textit{Pseudomonas aeruginosa} ATCC 27853, \textit{Shigella dysenteriae} ATCC 13313, \textit{Salmonella typhimurium} ATCC 43971, \textit{Staphylococcus aureus} ATCC 25923, \textit{Vibrio cholerae} ATCC 11623 and \textit{Candida albicans} CIP3153A by the twofold serial broth microdilution assay in concentration ranging from 5 mg/ml to 100 µ g/ml.
A phytochemical screening of alkaloids, polyphenols, terpenes, cardiac glycosides and other steroids were performed by TLC.

Only the leaves' ethanol extract showed some activity against *Vibrio cholerae* (MIC = 2.5 mg/ml) and *Staphylococcus aureus* (MIC = 1.25 mg/ml). The phytochemical screening of this extract revealed the presence of quercetin and caffeic acid derivatives as the major compounds.

\[ \text{CAFFEIC ACID} \]

\[ \text{QUERCETIN} \]

*Melaleuca leucadendron*

Tony Balacs\(^1\)\(^2\) reports of a paper which was the result of a collaboration between the Czech Republic and Vietnam, constituents of cajuput oil were found to have activity against the pathogenic bacteria *Pseudomonas aeruginosa*, terpinen-4-ol and \(\alpha\)-terpineol were the most effective components.

Lozoya and Navarro (Biomedical Research unit in Traditional Medicine and Drug Development. Mexican Institute of Social Security. Xochitepec, Mor. Mexico). Arnasan and Kourany. Faculty of Science University of Ottawa. Canada. The results were given for the experimental evaluation of *Mimosa tenuiflora* part I: screening of its antimicrobial properties. The study concluded that *in vitro* a strong inhibition growth effect was observed in all the gram positive and gram negative organisms, yeasts and dermatophytes used. *Pseudomonas aeruginosa* was amongst the cultures which were examined:-

*Citrus paradisi*

Lok (Univ. of Malaya) found Grapefruit seed extract *Citrus paradisi* (?) gave effective kill after 30 minutes at the following concentrations: *Pseudomonas aeruginosa* (100ppm). The kill time at the same concentrations was greater than 30 mins. The active component is naringenin. This chemical is found in various forms throughout the *Citrus* spp. These compounds are naringin, hesperidin, diosmin and naringenin.
Thus one would expect most of the citrus fruits to exhibit some degree of antimicrobial activity, if properly extracted.

Melaleuca alternifolia

Manufacturing Chemist\textsuperscript{11} looked at an investigation of the antimicrobial activity of p-cymene. For \textit{Pseudomonas aeruginosa}, the activity of p-cymene was found to be higher than that of the standard tea tree oil and more significantly also of terpinene-4-ol. Although p-cymene is usually only present at 2-5\% in commercial tea tree oil, its powerful antimicrobial activity makes a significant contribution to the oil's overall activity.

In a data sheet from Ateol (through Paroxite), we read that \textit{Melaleuca alternifolia} contains 1,8-Cineole (4 +/- 2\%) and terpinen-4-ol > 35\%. It is obtained from Lismore, Northern New South Wales.

The MIC (minimum inhibitory concentrations) against most commonly encountered pathogenic Gram negative and Gram positive bacteria and fungi are typically in the range 0.5 - 1.0\% v/v.

A typical gas chromatogram showed $\alpha$-thujene and $\alpha$-pinene, $\alpha$-terpinene, 1,8-cineole, gamma-terpinene, $\alpha$-terpinolene and terpinene-4-ol.

The report showed that Tea Tree oil should be used at 2\% to kill \textit{Pseudomonas aeruginosa}.

\textit{Aloe barbadensis} Miller
J.M. Marshall\textsuperscript{21} reports that Aloe vera gel is reported to be active against \textit{Pseudomonas aeruginosa}. Other studies have found the antibacterial activity to be limited to the sap drained from the leaves. According to Cera et al. (1980) While Aloe vera treatment was being carried out on dogs, biopsy samples were taken to test for \textit{Pseudomonas} infection and to determine prostaglandins and thromboxanes by an immuno-histological technique. Infection by \textit{Pseudomonas aeruginosa} was found to have been inhibited.

Soeda et al.\textsuperscript{22} found that an ointment containing 5\% Aloe was an effective treatment for trichophytiasis, and "Aloe Juice" was found to have inhibitory action against some bacteria and fungi, in particular \textit{Pseudomonas aeruginosa}, this is also confirmed by D. G. Spoerke\textsuperscript{20}.

Lee M. Cera, John P. Heggers, Martin C. Robson, William J. Hagstrom\textsuperscript{23}. Two case histories were presented where a therapeutic modality employing an Aloe vera cream (Dermaide Aloe) and tablets, reversed the dermal ischemia of burns due to prostaglandins and abrogated a \textit{Pseudomona aeruginosa} infection in animals with over a 35\% burn.

\section*{III ESCHERICHIA COLI}

\textit{Oxycoccus quadripetalus}

In the Lawrence Review\textsuperscript{6} of natural products (August 1987 reissued July 1994) we read that it does not appear to be that good a urinary tract disinfectant. However, one promising avenue is the use of the juice as a "urinary deodorant", since the acidity is sufficient to inhibit the growth of \textit{E. coli} in urine.

\textit{Cryptolepis sanguinolenta} Schltr.
\textit{Cryptolepis obtusa} N.E.Brown

Paolo, Houghton, E. Gomes et al\textsuperscript{2} report that species of \textit{Cryptolepis} are used in traditional African medicine for a variety of purposes. The MIC of the ethanolic and petroleum ether extracts were determined for \textit{Escherichia coli} ATCC 25922.

\textit{Nigella sativa}

In Research Reports\textsuperscript{24}. In a Bangladeshi paper, essential oil obtained from \textit{Nigella sativa} seeds was tested in vitro against four \textit{Shigella} species, against several strains of the organism responsible for cholera, \textit{Vibrio cholerae} and against \textit{E.coli} strains.

\textit{Plumbago zeylanica}

As reported earlier, the roots are vesicant and counter-irritant. They contain a crystalline principle, plumbagin or plumbagol, a 2-methyl-5-hydroxy-1,4-naphthoquinone, which has vitamin K-action and antibacterial properties. In a concentration of 1/50,000 plumbagin has a marked antibiotic action towards staphylococci and certain pathogenic fungi (\textit{Coccidioides imminites}, \textit{Histoplasma capsulatum}, \textit{Trichophyton ferrugenium}). Intravenous injections in patients with boils,
anthrax or cystitis were well tolerated and brought about rapid recovery (St. Rat and Luteraan, C.R. Acad. Sc., 1947, 224, 1587-89; St. Rat et al., Bull. Acad. Med., 1946, 130, 57-60; Bull. Acad. Nat. Med., 1948, 125-8). In vitro, the growth of Staph. aureus, Streptococcus pyogenes and Pneumococcus was completely inhibited at 1:100,000, of Myc. tuberculosis at 1:50,000 and of E. coli and Salmonella at 1:10,000 (Skinner33).

Melaleuca leucadendron

Tony Balacs12 reports on a paper which is the result of a collaboration between the Czech Republic and Vietnam, constituents of cajuput oil were found to have activity against the pathogenic bacteria Escherichia coli.

1,8-cineole, (-)-linalool, (-)-terpinen-4-ol and α-terpineol were all very active against E. coli in vitro.

Cuminum cyminum

Tony Balacs26 says that in an Indian study from Rajasthan, the steam distilled essential oil of cumin seed was found to have in vitro effectiveness against the bacteria Enterobacter cloacae and E.coli.

Aloysia triphylla

The Lawrence review of Natural products6 (Jan 1994) refers to Lemon verbena as Aloysia triphylla (L'Her.) Britt. Formerly described as Aloysia citriodora (Cav.) Ort., Verbena citriodora Cav., Verbena citriodora (Ort.) HBK: Family: Verbenaceae.

Chemistry: An essential oil, which is present in small quantities (0.42% to 0.65%), is extracted from the leaves by steam distillation. Known as oil of verbena, it contains a variety of fragrant compounds including citral (35%), methyl heptenone, carvone, l-limonene, dipentene and geraniol.

The essential oil is said to be acaricidal and bactericidal. An alcoholic leaf extract has been reported to have antibiotic activity in vitro against Escherichia coli.

Cinnamomum zeylanicum

Leung13 in his book says that cinnamon oil has antifungal, antiviral, bacteriacidal and larvicidal properties. A liquid carbon dioxide extraction at 0.1% has been demonstrated to suppress the growth of many organisms including E. coli.

Berberis vulgaris

In the Lawrence review of natural products6 (July 1991)
The wood and root are rich in isoquinoline alkaloids including palmatine, berbamine, oxyacanthine, jatrorrhizine, bervulcine, magnoflorine and columbamine.

However, the most important alkaloid is berberine. The root may contain as much as 3% alkaloids, which impart a yellow colour to the wood.

Berberine and several related alkaloids have been shown to have bacteriacidal activity, which in one study exceeded that of chloramphenicol (eg, Chloromycetin) against *Staphylococcus epidermidis*, *Neisseria meningitidis*, *Escherichia coli* and other bacteria.

*Santalum album*

*Glossogyne pinnatifida*

Richard Corbett\(^2\), reports that the essential oils of heartwood of *Santalum album* and of the whole of *Glossogyne pinnatifida* exhibited antibacterial activity against some pathogenic bacteria such as *Bacillus mycoides* and *Escherichia coli*.

*Pelargonium odorantissimum*
Pelargonium graveolens

S. Deans gives a study of the antibacterial action of essential oils, where geranium oil was found to be one of the top ten (out of 50) oils with regard to their inhibitory properties at a concentration of 1:10. It was active against twenty-one aerobes, E. coli, Pseudomonas and Streptococcus faecalis.

Achillea ageratum, Cephalophora aromatica, Rosmarinus officinalis, Tagetes signata, Aloe arborescens

Davidyuk, L.P., Lykov, I.N., Plakhova, N.S. In a search for antiseptics for the food and canning industries, the antimicrobial activity of 31 plant species and cultivars was tested on various microorganisms. Satureja montana, Helichrysum italicum, Rosmarinus officinalis and Coix lacrima [C. lacryma-jobi] were promising for providing antibiotic preparations. Antimicrobial activity in most plants was bacteriostatic. The bacteriostatic concentrations of plant preparations were determined in relation to Bacillus anthracoides [B. anthracis], Escherichia coli 1257 and Staphylococcus aureus. Preparations from Achillea ageratum, Cephalophora aromatica [Helenium aromaticum], Rosmarinus officinalis, Tagetes signata [T. tenuifolia] and Aloe arborescens were bacteriostatic at 62.5 µg/ml.

Cassia obtusifolia L.

Kitanaka, S., Takido, M. Anthraquinones (islandicin, helminthosporin, chrysophanol, physcion, xanthorin, 8-O-methylchrysophanol, obtusifolin, emodin and aloe-emodin), a benzoquinone (2,5-dimethoxybenzoquinone), a naphtho-gamma-pyrone (rubrofusarin), phytosterols and betulinic acid were isolated from the roots. Aloe-emodin and 2,5-dimethoxybenzoquinone from the roots, and isotoralactone, toralactone, questin and torosachrysone from the seeds showed antimicrobial activity against Staphylococcus aureus and Escherichia coli.

Cumin cyminum

Tony Balacs in his Research Reports.

Singh and Upadhyay, working in Gorakhpur, India, showed that cumaldehyde, the main constituent of cumin seed oil was strongly fungitoxic against Aspergillus flavus as well as A. niger.
Whole cumin oil inhibited both species of *Aspergillus* by over 90% when at 2000ppm and by 100% at 3000ppm. When the aldehyde fraction of the oil (containing cumaldehyde) was tested alone, it was found to have all the antifungal activity (at least 85% inhibition at 500ppm), whereas the residual oil was entirely inactive. (0% at 3000ppm).

Tony Balacs in Research Reports. In an Indian study from Rajasthan, the steam distilled essential oil of cumin seed was found to have in vitro effectiveness against the fungi *Aspergillus flavis*.

*Plumbago zeylanica*

In a file from Dr Stephen Greenburg we learn that the root has been shown to contain plumbagin, a yellow naphthoquinone, which is responsible for its antimicrobial and antibiotic activity. (ref. Bep Oliver-Bever and N. Atkinson & H.E. Brice).

A very dilute solution (i.e. a concentration of 1:50,000) of plumbagin is lethal to a wide spectrum of bacteria and to pathogenic fungi, i.e. *Coccidiodoides imminitis*, *Histoplasma capsulatum*, *Trichophyton* spp., *Candida albicans*, *Aspergillus niger* and *A. flavus*. (ref. F.A. Skinner).

*Cinnamomum camphora*

Balacs in Research Reports. It has been shown in a paper from India that the essential oil of *Cinnamomum camphora* strongly inhibits the growth of *Aspergillus flavus*, a common toxin-producing fungus, which grows during the storage of food. Oil of camphor is effective at a concentration of 4 parts per thousand and moreover, is as potent as some synthetic preservatives (dithane, copper oxychloride and thiovit).

The oil is equally effective against *Aspergillus sulphures* three species of *Curvularia*, four of *Fusarium* and against *Penicillium citrinum* (the mould which spoils lemons). However, *Aspergillus fumigatus*, *niger*, parasiticus and *terrei* all appear to be resistant to the oil at 5 parts per thousand.

*Usnea barbata*
Aspergillus niger ATCC 1015 was inhibited at 2.7-8.2 µg/L and Aspergillus flavous ATCC 9643 at 0.9-2.7 µg/L.

Cymbopogon citratus
Adropogon citratus

Grace O. Onawunmi\textsuperscript{34}, says that the antifungal activity of lemongrass oil has been evaluated using fungistatic (MIC and agar diffusion tests) and fungicidal (spore germination) studies. Appreciable activity was observed against various isolates of Candida and clinical isolates of Aspergillus fumigatus, Microsporum gypseum and Trichophyton mentagrophytes. The most resistant organism was A. fumigatus while M. gypseum and the Candida spp were the most susceptible of the isolates. Comparative studies with pure samples of citral and citronellal, constituents of lemongrass oil, showed good activity against the test fungi while dipentene and myrcene showed no activity.

Exposure of the spores of A. fumigatus to 0.1% lemongrass oil for five minutes resulted in 93% of spores not germinating while lower concentrations (0.08% and 0.05%) caused 80% and 60% reductions in spore germination respectively. Challenge tests showed that 0.25% lemongrass oil in an aqueous cream would effectively preserve it against fungal contamination.

Tony Balacs in Research Reports\textsuperscript{1}. A research group from Lahore, Pakistan, has been studying the inhibitory effects of lemongrass oil (Cymbopogon flexuosus) against pathogenic fungi.

The samples of oil were either from local or from Thai lemongrass; all contained between 70% and 80% citral. No significant differences in activity or selectivity for particular fungi were found between the oil samples, although the oil with the highest citral concentration was the most active.

The following fungi were screened: Aspergillus niger, A. fumigatus, Candida albicans, Trichophyten tonsurance (all isolated from patients); A. parasiticus, Penicillium digitatum, Helminthporium oryzae (all isolated from plants); Monilia sitophilia (from seeds); and Saccharomyces cerevisiae (from food). M. sitphilia was inhibited by lemongrass oil at a concentration of 500 parts per million in vitro. P. digitatum at 1500 ppm. and A. niger and A. fumigatus at 2000 ppm. these concentrations represent the lowest levels of oil at which in vitro inhibition was seen.

Litsea cubeba

Tony Balacs\textsuperscript{31} in his Research Reports. Moleyar and Narasimham, working in Mysore, India, have found that citral, a mixture of geranial and neral found in Litsea cubeba, Melissa and lemongrass is active in inhibiting the growth of the common fruit fungus, Aspergillus niger, being superior to camphor.

Balacs\textsuperscript{4} says that May chang is similar chemically to lemongrass, melissa and other essential oils rich in citral, and its therapeutic properties are similar to lemongrass. It is used as a commercial source of citral. Citral accounts for 75% of may chang oil and has
two isomers which are neral and geranial, which are the respective aldehyde equivalents of nerol and geraniol. May chang oil contains slightly more geranial (41%) than neral (34%). Citral is known to be antitumoral and antifungal.

The antifungal effects of citral and lemongrass oil were published in IJA Vol.3 No.1. Citral showed significant action against *Microsporum gypseum*, *Aspergillus fumigatus*, *Trichophyton mentagrophytes* var. *interdigitale*, and *Candida albicans*.

*Melaleuca alternifolia*

In the Lawrence review of Natural products (Jan 1991)

Following steam extraction, the leaves approximately a pale yellow oil, with a pleasant terpenic odour. The oil 50% to 60% terpenes (pinene, terpinene, cymene), from 6% to 8% cineol and a variety of minor sesquiterpenes and related alcohols.

The oil was found to have an MIC of 0.5% v/v for *Aspergillus niger* and *Trichophyton*, and 0.025% v/v for *Candida albicans*.

*Aloe barbadensis* Miller

Ahmad, S., Kalhoro, M.A., Kapadia, Z., Badar, Y. Occurrence of Aloe spp., phytochemical analysis and uses of commercial "aloe" are discussed. Traditional, medicinal, biological (including activity against bacteria, *Aspergillus niger* and *Trichophyton mentagrophytes*), cosmetic, and food and industrial uses are covered.

V STAPHYLOCOCCUS AUREUS

*Drosera rotundifolia*

Hoffmann refers to *Drosera rotundifolia* and says that the entire plant is used. It contains naphthaquinones inuding plumbagin; flavonoids; tannins; citric and malic acid. It is antispasmodic, demulcent, expectorant. Sundew may be used with great benefit in bronchitis and whooping cough. The presence of plumbagin helps to explain this, as it has been shown to be active against streptococcus, staphylococcus and pneumococcus bacteria. Sundew will also help with infections in other parts of the respiratory tract.

*Alkanna tinctoria*


Its bark and roots are rich in pigments of a naphthaquinone structure. They are acetic, β,β-dimethylacrylic, isovaleric, angelic, and β-acetoxy-isovaleric esters of alkannin, an alcohol discovered by Brockmann, which seems to be an artifact hydrolytic product; deoxyalkannin is also present.
It exhibits antibiotic activity against *Staphylococcus*. The antimicrobial activity of an n-hexane root extract appears to be attributed to the naphthaquinone pigments. Alkannin isovalerate and angelate have been used for treating ulcer crucis patients. Compositions with alkannin derivatives improve healing of leg ulcers connected with varicosis, and particularly improve wound granulation and epithelialisation tendency.

**Perilla frutescens**

Tony Balacs\(^1\). A Californian group has found that *Perilla frutescens* (Shiso oil) which contains perillaldehyde, (74%) and limonene (12.8%) has antimicrobial activity, mainly due to the perillaldehyde. Perillaldehyde inhibits fungi and both Gram-positive and Gram-negative bacteria.

In this study, perilla oil was especially effective against *Acnes propionibacterium* and *Staphylococcus aureus* (both of which can cause acne).

**Artemisia tridentata** Nutt

Francis Brinker\(^3\) says that the advantage of volatile inhalation in respiratory infections has been challenged on the basis that it actually impairs normal immune defences. This was studied with a compound similar to A.tridentata oil which included camphor, turpentine spirits, eucalyptus oil and thuja oil, as well as menthol and thymol. However, it was shown that using this same compound did not impair either mucociliary or phagocytic function after four- or eight-hour exposures. In fact, exposure to these vapours before and after challenge by the infectious bacterial agent (*Staphylococcus aureus*) significantly reduced the number of viable organisms remaining after four hours. This refutes the earlier claim and suggests the advantage of inhalation during a viral upper respiratory infection in order to prevent complications from a secondary bacterial invasion. Furthermore, an alpha- and beta-pinene oxidation product has also been shown to enhance the activity of tetracycline against *Diplococcus pneumoniae* and four other common bacteria by acting as a vehicle and causing greater cytopermeability, as well as being bacteriostatic itself.

![alpha-PINENE](attachment:alpha-PINENE.png)

**Schizandra chinensis**

In a data sheet\(^4\) we read of *Schizandra chinensis* where the fruit is used.

Schizandra has been shown to have activity against mycobacteria, *Staphylococcus aureus* and others. Extracts of Schizandra are able to induce non-specific resistance in man similar to the effect of ginseng.

**Persea americana**
The Lawrence review of natural products (April 1993) refers to Avocado as *Persea americana* Mill. [Syn. *Persea gratissima* Gaertn.] also referred to as *Laurus persea*.

Several of the unsaturated oxygenated aliphatic compounds in the pulp and seed have been shown to possess strong in vitro activity against gram-positive bacteria, including *Staphylococcus aureus*.

**Calendula officinalis**

I. Morelli, E. Bonari, A.M. Pagni and P.E. Tomei, also F. Menichini. Its flowers are rich in triterpenoid and steroidal compounds: α- and β-amyrin, lupeol, theta(w)-taraxasterol, erythrodiol, brein, faradiol, arnidiol, calenduladiol, ursadiol, maniladiol, helantriols B0, B1, B2, and A1, longispinogenin, urs-12-en-3,16,21-triol, oleanolic acid, calendulosides C, D, E, F, G, and H; sitosterol, stigmasterol, campasterol, cholesterol, and their derivatives. It contains essential oil (caryophyllene, calephlone, menthone, isomenthone, terpenic hydrocarbons); carotenoids and β-carotene; p-hydroxybenzoic, protocatechic, gentizic, vanillic, p-coumaric, caffeic, and ferulic acids; vitamin E and polypropenyl quinones; flavonoid compounds (isorhamnetin and quercetin glycosides).

It has been proved to have aromatic, anti-haemorrhagic, emmenagogue, styptic, antiseptic, anti-inflammatory, vulnerary, spasmolytic, diaphoretic, and cholagogue activities. Moreover, it has vasoprotective action and antibacterial activity, particularly against *Staphylococcus aureus*.

**Matricaria chamomilla**

Leung refers *Matricaria chamomilla*, which has been reported to have numerous pharmacological properties, some of which are the following: The oil has bactericidal and fungicidal activities, particularly against Gram-positive bacteria (e.g. *Staphylococcus aureus*) and *Candida albicans*. It also reduced blood urea concentration in rabbits to a normal level.

**Terminalia avicennioides** Guill. & Perr.

Application of powdered, ground roots or root-bark used. The leaves are applied to the skin to prevent inflammation. The powdered root is also applied to sores and ulcers

In Casamanance of Senegal the root bark is considered cleansing and healing on refractory sores, according to Kerharo and Adam.

Though the roots are used as chew-sticks in the Ibadan area of Nigeria, they have no antibiotic activity, however examination of the roots for their use in the treatment of skin infections showed activity against a number of Gram +ve organisms, including *Staphylococcus aureus*. Lewis and Elvin-Lewis say that the root is used in West Africa for the treatment of wounds and that it produces no adverse clinical effects when used as a chewing stick. However, aqueous extracts have been shown to have antibacterial activity.
REFERENCES


5. Dr Stephen Greenburg (Lipo Chemicals Inc.) thesis entitled "Ethnic Botanical Literature" author anon.

6. The Lawrence review of Natural Products. Copyright 1994 by Facts and Comparisons (ISSN 0734-4961). 111 West Port Plaza Suite 400, St. Louis, Missouri 63146-3098.


25. Bep Oliver: Medicinal Plants in Nigeria - being a course of four lectures delivered in April 1959 in the Pharmacy Department of the Nigerian College of Arts, Science and Technology, Ibadan. Published as a private edition 1960 by the Nigerian College of Arts, Science and Technology.


33. F.A. Skinner. "The antibiotics" In Modern Methods of Plant Analysis (Eds. K. Peach and H.V. Tracy). Published Springer-Verlag, West Germany, 3:626-725.


CHEMICAL COMPOSITION OF PLANT MATERIALS

What are these constituents and, can we obtain them? If we can obtain them are they legally permitted? In some cases the answer is definitely "Yes", we can extract and legally use benzoic acid and benzyl alcohol, both of which are the subject of pharmaceutical monographs and listed in permitted section of the cosmetic legislation. Be prepared to pay anything from £400 to £800/Kg or more!

The sources in these cases are from Benzoin Siam and Tolu Balsam.

Another source of preservative comes from a commercially available Grapefruit seed extract which has a number of benefits
Aloe Vera is a source of p-hydroxy cinnamic acid.

Potassium sorbate or any sorbate salt, such as that from the Rowan berry - a rich source of sorbic acid and sorbitol (Merck) is well worth a look.

Appendix I

DEFINITIONS USED TO DESCRIBE MATERIALS ACTIVE AGAINST SPOILAGE ORGANISMS.

**ANTIBIOTIC** - inhibiting the growth of another organism, used especially of a substance produced by micro-organisms which, in dilute solution, has the capacity to inhibit the growth of, or to destroy, micro-organisms causing infectious diseases.

**ANTIMICROBIAL** - lit. against a microscopic organism, especially a disease causing bacterium.

**ANTIFUNGAL** - lit. against a fungus.

**ANTISEPTIC** - an agent that causes the destruction, or inhibition of growth, of bacteria.

**BACTERICIDE** - a substance that destroys or is capable of destroying bacteria.

**FUNGICIDE** - a means of killing fungi.

**PRESERVATIVE** - a safeguard, a prophylactic, serving to preserve.

**PROPHYLACTIC** - guarding against disease, a preventive of disease.

**GERMICIDAL** - that which kills germs (a rudimentary form of a living thing, whether plant or animal - a micro-organism, especially a malign one).

**DISINFECTANT** - anything which destroys the causes of infection (where an infection can include diseases, pathogenic micro-organisms).

Appendix II

GENERAL SPOILAGE CLASSIFICATIONS

Moulds or molds
Yeast
Bacteria
Bacilli
Spoilage (organisms)
ORGANISMS FREQUENTLY MENTIONED IN THE LITERATURE

Candida albicans
Pseudomonas aeruginosa
Escherichia coli
Staphylococcus aureus
Aspergillus niger
Streptococcus spp
Enterobacter spp
Penicillium spp
Curvularia spp
Bacillus subtilis
Saccharomyces cerevisiae
Drechslera sp

Appendix III
SEARCH ON "FUNGAL", "FUNGICIDAL" OR "FUNGICIDE"


Appendix IV
SEARCH ON "BACTERICIDE", "BACTERICIDAL"

Abelmoschus moschatus Medic., Allium odorum, Allium sativum, Aloe barbadensis, Aloysia triphylla, Anthemis nobilis, Artemisia absinthium, Bellis perennis, Berberis vulgaris, Calendula officinalis, Canarium luzonicum, Caryophyllus aromaticus, Centella asiatica, Cetraria islandica, Cinnamonium zeylanicum, Citrus paradisi, Citrus limonum, Citrus medica, Citrus racemosa, Citrus decumana, Eucalyptus globulus, Eugenia caryophyllata, Eugenia aromatica, Eupatorium fortunei, Gentiana lutea, Ginkgo biloba,
Hibiscus abelmoschus, Hippophae rhamnoides, Humulus lupulus, Hydrocotyle asiatica,
Inula helenium, Jambosa caryophyllus, Lithospermum erythrorhizon, Matricaria officinalis,
Mauritia flexos>, Malaleuca alternifolia, Monotropa uniflora,
Myrica cerifera, Persea americana, Phellodendron amurensis, Propolis, Prostanthera
stratiophora, Salvia officinalis, Syzygium aromaticum, Taraktogenos kurzii King, Thymus
vulgaris, Usnea barbata, Valeriana officinalis, Verbasca thapsus

Appendix V

SEARCH ON "DISINFECT" OR "DISINFECTANT"

Agave americana, Allium sativum, Allium cepa, Aloe barbadensis, Aloe vera,
Arctostaphylos uva-ursi, Artemisia abrotanum, Artemisia tridentata Nutt, Ascophyllum
nodosum, Aster tataricus L., Aster tataricus L., Baphia nitida, Betula pendula, Betula
alba, Calendula officinalis, Calluna vulgaris, Carum carvi, Caryophyllus aromaticus,
Chrysanthemum parthenium, Cinchona succirubra, Citrus decumana, Citrus paradisi,
Citrus racemosa, Cochlearia officinalis, Commiphora myrrha, Commiphora molmol,
Cupressus sempervirens, Eucalyptus globulus, Eugenia caryophyllata, Eugenia
aromatica, Fagara capensis, Humulus lupulus, Hypericum perforatum, Jambosa caryophyllus, Juglans regia, Juniperus communis, Kigelia africana,
Lavandula officinalis, Lavandula angustifolia, Lygodium circinnatum (N.L.Burm.)
Swarz, Magnolia glauca, Majorana hortensis, Majorana onites, Melaleuca alternifolia,
Melissa officinalis, Mentha piperita, Origanum heracleoticum, Origanum vulgare,
Origanum onites, Origanum majorana, Oxycoccus quadripetalus, Petasites vulgaris,
Pyrola minor, Salix vitellina, Salvia officinalis, Santalum album, Saponaria officinalis,
Solidago virgaurea, Symphytum officinale, Syzygium aromaticum, Tabernaemontana
crassa, Tanacetum vulgare, Tanacetum parthenium, Taraktogenos kurzii King,
Taraxacum officinale, Thymus vulgaris, Tropaeolum majus, Tussilago petasites,
Umbellularia californica [H. & A.] Nutt., Vaccinium vitis-idaea, Zanthoxylum capense

Appendix VI

SEARCH ON "GERMICIDAL" OR "GERMICEDE"

Andira araroba, Betula alba, Betula pendula, Carum coticum, Carum ajowan, Citrus
mitis, Citrus microcarpa Bge.(by Tanaka), Humulus lupulus, Lavandula officinalis,
Lavandula angustifolia, Lithospermum erythrorhizon, Malaleuca alternifolia,
Phellodendron amurencis, Sclerocarya birrea subsp. caffra, Thymus vulgaris,
Trachyspermum ammi, Vaccinium myrtillus

Appendix VII

SEARCH ON "ANTISEPTIC"

Abies cilicia, Achillea millefolium, Adropogon citratus, Agathosma betulina, Agave
americana, Ajuga spp, Alkanna tinctoria, Alliaria petiolata, Allium sativum, Allium cepa,
Aloe barbadensis, Amyris balsamifera, Anthemis nobilis, Anthriscus sylvestris,
Anthriscus cerefolium, Anthyllis vulneraria, Apium graveolens, Aquilegia vulgaris,
Arbutus unedo, Arctium lappa, Arctostaphylos uva-ursi, Armeria maritima, Armoracia
rusticana, Arnica montana, Artemisia tridentata Nutt, Artemisia absinthium, Artemisia

Appendix VIII

SEARCH ON "ANTIBIOTIC"


Appendix IX

SEARCH ON "ANTIMICROBIAL"

officinale, Lippia chevalieri Moldenke, Matricaria officinalis, Melaleuca alternifolia, Melissa officinalis, Mimosa tenuiflora, Nymphaea alba major aquatica, Nymphaea candida, Ocimum sanctum, Ocimum basilicum, Passiflora incarnata, Pavetta oblongifolia (Hiern) Bremek, Pelargonium odorantissimum, Pelargonium graveolens, Pentaglottis sempervirens, Petroselinum crispum, Piliostigma thonningii, Pinus silvestris, Piper methysticum, Plantago major, Plumbago zeylanica, Podalyria tinctoria, Rosmarinus officinalis, Salvia hispanica, Salvia officinalis, Sanguinaria canadensis, Sanguisorba officinalis, Satureia montana, Satureia hortensis, Sophora tinctoria, Terminalia macroptera, Terminalia glaucescens, Terminalia ivorensis, Terminalia avicennioides, Teucrium scorodonia, Teucrium chamaedrys, Thymus vulgaris, Verbena officinalis.