

"NATURAL PRESERVATIVES"

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SUMMARY

This paper looks at the theoretical development of a natural preservative system using the author's data base 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:-

Annex VI Part 1 or 2 of the EEC Cosmetic Directive 76/768/EEC - including 7th. amending Commission Directive 94/32/EC.

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 desiccated 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 micro-organisms.

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 simulataneously 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 Balacs¹ reported that Savory, calamintha and thyme were all very active in vitro against *Staphylococcus aureus*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Candida albicans*.

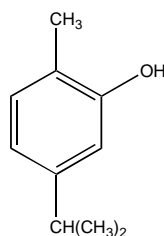
Cryptolepis sanguinolenta Schltr.
Cryptolepis obtusa N.E.Brown

Alexandra Paulo, Aida Duarte, Peter Houghton and Elsa Gomes² reported that species of *Cryptolepis* are used in traditional African medicine for a variety of purposes. The roots and leaves decoction of *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 *Candida albicans* CIP3153A by the twofold serial broth microdilution assay in concentration ranging from 5 mg/ml to 100 µg/ml.

Satureia hortensis
Satureia montana

Valnet³ says that *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 Montpellier did a study on the antibacterial and antifungal properties. 10 types of *Staphylococcus*, 14 other microorganisms and 11 fungi were examined including *Candida albicans*, *C. tropicalis*, *Trichophyton interdigitalis*. The results were very encouraging. In this respect it was equal to thyme in performance.



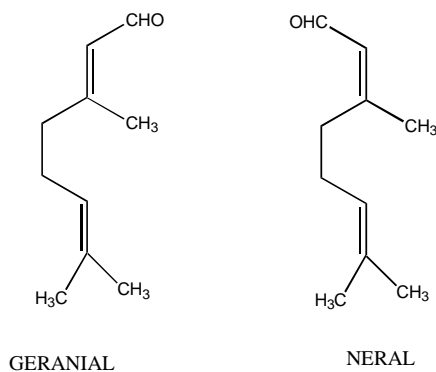
CARVACROL

Tony Balacs¹ says that savory oil was rich in carvacrol (56.8%), and that it was very active in vitro against *Candida albicans*.

Litsea cubeba

Tony Balacs⁴, says that *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

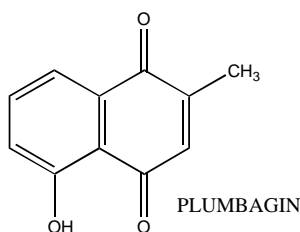
on 125 people with cancer. In six (5%) cases a ten-year follow up showed complete cure. Full report IJA Vol.1, No.4 / Vol.2, No.1.



The antifungal effects of citral and lemongrass oil were published in IJA Vol.3 No.1. Citral showed significant action against *Microsporium 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 immitis*, *Histoplasma capsulatum*, *Trichophyton* spp., *Candida albicans*, *Aspergillus niger* and *A. flavus*. (F.A. Skinner³³).

Lapacho colorado

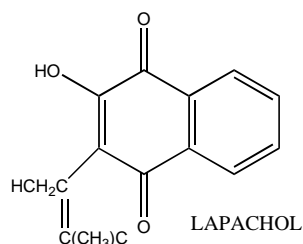
Lapacho morado

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 led 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 tabebuina. 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 Home⁷ 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 decrease: alpha-terpinene, gamma-terpinene, terpinolene, terpinen-4-ol, and as the following compounds increase: cineole, limonene, alpha-terpineol.

The Lawrence review⁶ 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*.

Price⁸ says it is also used for colds, mouthwash and sore throat, for bronchitis, candida, infected wounds and insect bites.

Rosalind Blackwell⁹ 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 paracymenthene 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, Saras¹⁰ report that the antimicrobial activity was discovered in 1920's when Penfold and Grant reported that the essential oil extracted from *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 superseded 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 *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.

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 *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 Chemist¹¹. 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 *Candida albicans*. However, there was no simple correlation between terpinene-4-ol levels of the oils and their activity against *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 *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 *Staphylococcus* the terpinene-4-ol. For *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 Balacs¹² reports that 1,8-cineole, (-)-linalool, (-)-terpinen-4-ol and α -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 α -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

Leung¹³ 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*, *Staphylococcus aureus*, and *Candida albicans*.

Usnea barbata

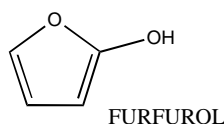
In a paper by James and Mitchell¹⁴ presented at a symposium in London in we read that Alpine Lichen is cited as a source of Usnic acid, which was first extracted in 1843 by Rochleder and Heldt, and it is chemicaly dibenzofuran or 6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3-(2H,9bH)-dibenzofuran-1-one.

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

Helichrysum angustifolium DC.

Leung¹³ 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, β -pinene, and furfural.



Pinus silvestris

Research Reports¹⁵. 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 Baxter¹⁶ 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*, *Microsporium 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 products⁶ (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

Trattler¹⁷ lists numerous benefits of garlic, including yeast infections (*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 sulphoxide. 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 (*Candida*).

Echinacea angustifolia

Glenise McLaughlin¹⁸ gives a long list of indications which includes the treatment of *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 *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 *Echinacea* cream and liquid extract and a cream alone. The extract was more effective than the cream alone.

Propolis

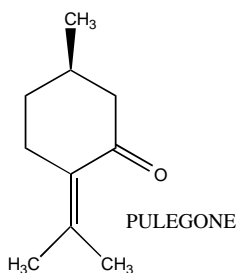
Allan Onions¹⁹ talking of propolis. Early work on propolis suggested widespread antibacterial activity, but more recent studies have confirmed that the activity is restricted to certain bacteria, with good results having been recorded against *B. mesentericus*, *M. lysodeicticus*, *P. vulgaris*, *S. aureus* and *Strept. cremoris*. Propolis does, however, show excellent antimycotic activity, particularly against *Candida albicans*, *E. inguinalis*, *E. rubrum* etc.

II PSEUDOMONAS AERUGINOSA

Calamintha nepeta

1. Tony Balacs¹ reports that an Italian group analysed the essential oils of four Mediterranean Lamiaceae for antimicrobial activity. *Satureia montana*, *Thymus vulgaris*, *Calamintha nepeta* and *Rosmarinus officinalis* oils were all found to be active.

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



Calamintha was moderately active against *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.

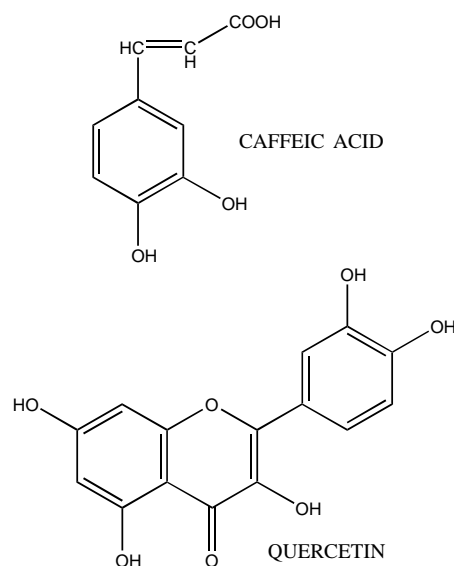
Cryptolepis obtusa N.E.Brown

Houghton and Gomes et al.² report that species of *Cryptolepis* are used in traditional African medicine for a variety of purposes. The roots and leaves decoction of *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 *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Shigella dysenteriae* ATCC 13313, *Salmonella typhimurium* ATCC 43971, *Staphylococcus aureus* ATCC 25923, *Vibrio cholerae* ATCC 11623 and *Candida albicans* CIP3153A by the twofold serial broth microdilution assay in concentration ranging from 5 mg/ml to 100 µg/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.



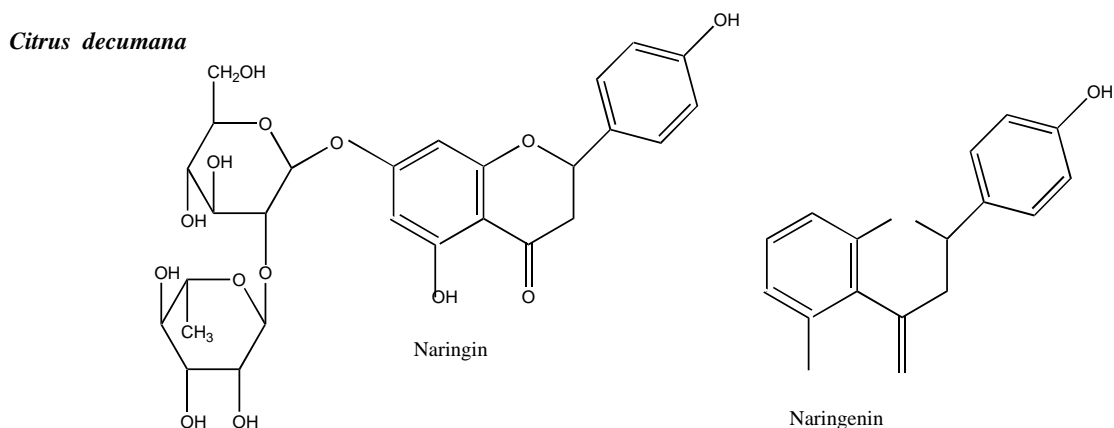
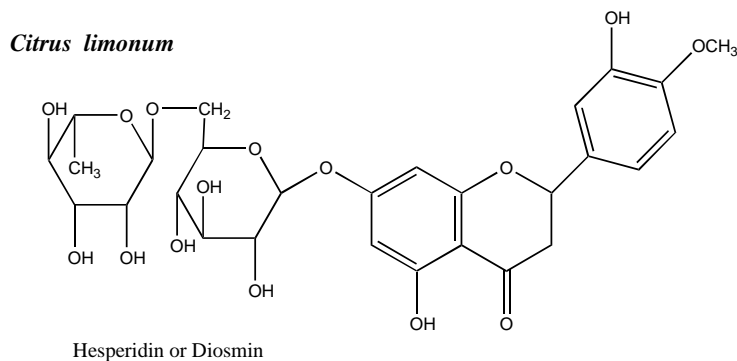
Melaleuca leucadendron

Tony Balacs¹² 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 *Pseudomona aeruginosa*, terpinen-4-ol and α -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. *Pseudomona 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 Chemist¹¹ looked at an investigation of the antimicrobial activity of p-cymene. For *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 *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 α -thujene and α -pinene, α -terpinene, 1,8-cineole, gamma-terpinene, α -terpinolene and terpinene-4-ol.

The report showed that Tea Tree oil should be used at 2% to kill *Pseudomonas aeruginosa*.

Aloe barbadensis Miller

J.M.Marshall²¹ reports that Aloe vera gel is reported to be active against *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 Pseudomonas infection and to determine prostaglandins and thromboxanes by an immuno-histological technique. Infection by *Pseudomonas aeruginosa* was found to have been inhibited.

Soeda et al.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 *Pseudomonas aeruginosa*, this is also confirmed by D. G. Spoerke²⁰.

Lee M. Cera, John P. Heggers, Martin C. Robson, William J. Hagstrom²³. 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 *Pseudomonas aeruginosa* infection in animals with over a 35% burn.

III ESCHERICHIA COLI

Oxycoccus quadripetalus

In the Lawrence Review⁶ 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 *E. coli* in urine.

Cryptolepis sanguinolenta Schltr. *Cryptolepis obtusa* N.E.Brown

Paolo, Houghton, E. Gomes et al². report that species of *Cryptolepis* are used in traditional African medicine for a variety of purposes. The MIC of the ethanolic and petroleum ether extracts were determined for *Escherichia coli* ATCC 25922.

Nigella sativa

In Research Reports²⁴. In a Bangladeshi paper, essential oil obtained from *Nigella sativa* seeds was tested in vitro against four *Shigella* species, against several strains of the organism responsible for cholera, *Vibrio cholerae* and against *E.coli* strains.

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 (*Coccidioides immitis*, *Histoplasma capsulatum*, *Trichophyton ferrugineum*). 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.

Cumin 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.

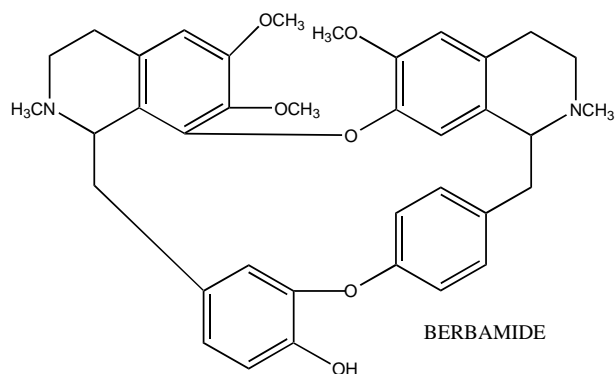
The essential oil is said to be acaricidal and bactericidal. An alcoholic leaf extract has been reported to have antibiotic activity in vitro against *Escherichia coli*.

Cinnamomum zeylanicum

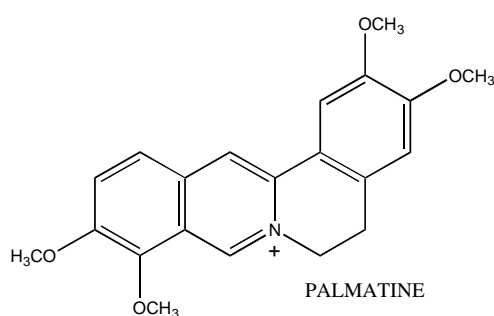
Leung¹³ in his book says that cinnamon oil has antifungal, antiviral, bacteriacidal and larvicidal properties. A liquid carbon dioxide extraction at 0.1% has been demonstrated to suppress the growth of many organisms including *E. coli*.

Berberis vulgaris

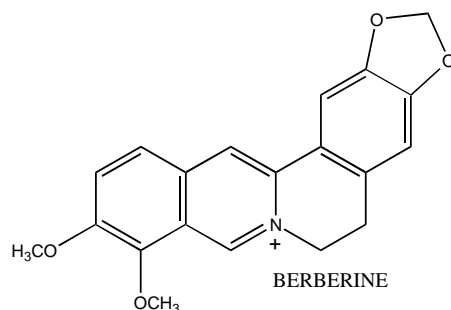
In the Lawrence review of natural products⁶ (July 1991)



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 bacteriicidal 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 Corbett²⁷, 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

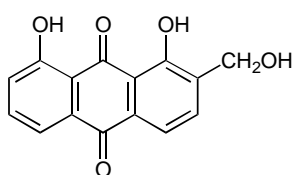
S.Deans²⁸ gives a study of the antibacterial action of essential oils, where geranium oil was found to be one of the top ten (out of 50) oils with regard to their inhibitory properties at a concentration of 1:10. It was active against twenty one aerogenes, *E. coli*, *Pseudomonas* and *Streptococcus faecalis*.

Achillea ageratum,
Cephalophora aromatica
Rosmarinus officinalis
Tagetes signata
Aloe arborescens

Davidyuk, L.P., Lykov, I.N., Plakhova, N.S.²⁹. 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.³⁰. 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 torosachryson from the seeds showed antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.



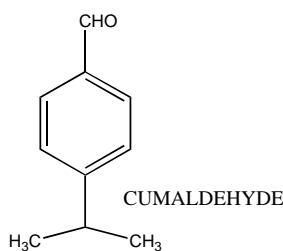
ALOE EMODIN

IV ASPERGILLUS NIGER and SPP.

Cumin cyminum

Tony Balacs³¹ 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*.

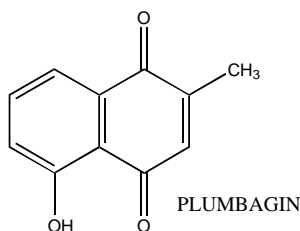


Whole cumin oil inhibited both species of *Aspergillus* by over 90% when at 2000ppm and by 100% at 3000ppm. When the aldehyde fraction of the oil (containing cumaldehyde) was tested alone, it was found to have all the antifungal activity (at least 85% inhibition at 500ppm), whereas the residual oil was entirely inactive. (0% at 3000ppm).

Tony Balacs²⁶ 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 flavus*.

Plumbago zeylanica

In a file from Dr Stephen Greenburg⁵ 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-Bever²⁵ and N. Atkinson & 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 immitis*, *Histoplasma capsulatum*, *Trichophyton* spp., *Candida albicans*, *Aspergillus niger* and *A. flavus*. (ref. F.A. Skinner³³).

Cinnamomum camphora

Balacs in Research Reports²⁴. 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 *terreus* 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. Onawunmi³⁴, 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 Reports¹. 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*, *Helminthorporium oryzae* (all isolated from plants); *Monilia sitophilia* (from seeds); and *Saccaromyces 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 Balacs³¹ 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.

Balacs⁴ 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 *Microsporium gypseum*, *Aspergillus fumigatus*, *Trichophyton mentagrophytes* var. *interdigitale* and *Candida albicans*.

Melaleuca alternifolia

In the Lawrence review of Natural products⁶ (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., Kalhor, M.A., Kapadia, Z., Badar, Y.³⁵. 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

Hoffmann³⁶ refers to *Drosera rotundifolia* and says that the entire plant is used. It contains naphthaquinones including 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

I. Morelli, E. Bonari, A.M. Pagni and P.E. Tomei, also F. Menichini³⁷: Selected Medicinal Plants. FAO Plant Production and Protection Paper 53/1. Food and Agriculture organisation of the United nations. Rome 1983. FAO 1984. ISBN No. 92-5-101481-7.

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 ulcus crucis patients. Compositions with alkannin derivatives improve healing of leg ulcers connected with varicosis, and particularly improve wound granulation and epithelialisation tendency.

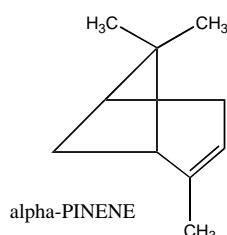
Perilla frutescens

Tony Balacs¹. 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 Brinker³⁸ 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.



Schizandra chinensis

In a data sheet³⁹ 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 products⁶ (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. Menichini³⁷. 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 polyprenyl 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*.

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 Adam⁴⁰.

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-Lewis⁴¹ 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.

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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"

Ajuga bracteosa Wall. ex Benth., *Aleurites moluccans*, *Allium sativum*, *Aloe barbadensis*, *Aloe vera*, *Anacardium occidentale*, *Anthemis nobilis*, *Arctium lappa*, *Argemone mexicana*, *Artemisia tridentata* Nutt, *Arthraxon hispidus* (Thunb.) Merr., *Arthraxon ciliaris* Beauv., *Arthraxon hispidus* (Thunb.) Merr., *Azadirachta indica*, *Barringtonia racemosa* (L.) Blume ex DC., *Bonafousia muelleriana* (Mart.) Boit. & L.Allorge, *Bonafousia undulata* (Vahl) A.DC., *Calendula officinalis*, *Canarium luzonicum*, *Carica papaya*, *Caryocar villosum* (Aublet) Pers., *Cassia alata*, *Cassia absus*, *Cassia occidentalis*, *Cassia tora*, *Cassuvium pomiferum*, *Celastrus angulatus* Maxim. (*C. latifolium* Hemsl.), *Cetraria islandica*, *Chelidonium majus*, *Chlorophora excelsa*, *Citrus sinensis*, *Citrus racemosa*, *Citrus decumana*, *Citrus bigaradia*, *Citrus paradisi*, *Commiphora molmol*, *Commiphora myrrha*, *Coriandrum sativum*, *Cumin cyminum*, *Curcuma amada*, *Cymbopogon citratus* (syn. *Adropogon citratus*), *Echinacea angustifolia*, *Eucalyptus globulus*, *Ficus racemosa*, *Geranium maculatum*, *Hirtella racemosa* Lam., *Iryanthera juruensis* Warb., *Jatropha multifida* Linné, *Jatropha curcas* Linné, *Juglans regia*, *Lavandula officinalis*, *Lavandula angustifolia*, *Lawsonia alba*, *Ligusticum sinense*, *Lygodium circinnatum* (N.L.Burm.) Swartz, *Majorana hortensis*, *Majorana onites*, *Matricaria officinalis*, *Melaleuca alternifolia*, *Origanum majorana*, *Origanum onites*, *Origanum vulgare*, *Origanum heracleoticum*, *Phytolacca decandra*, *Phytolacca americana*, *Phytolacca rigida*, *Pinus silvestris*, *Poria cocos*, *Prostanthera striatiflora*, *Rubus fruticosus*, *Salix babylonica* L., *Scutellaria baiacalensis*, *Thymus vulgaris*, *Trifolium pratense*, *Usnea barbata*, *Zanha africana*, *Zingiber officinale*.

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*, *Cinnamomum 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, Lippia citriodora, Lithospermum erythrorhizon, Matricaria officinalis, Mauritia flexosa, Melaleuca alternifolia, Monotropa uniflora, Myrica cerifera, Persea americana, Phellodendron amurense, Propolis, Prostanthera striatiflora, Salvia officinalis, Syzygium aromaticum, Taraktogenos kurzii King, Thymus vulgaris, Usnea barbata, Valeriana officinalis, Verbascum 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, Cupresses sempervirens, Eucalyptus globulus, Eugenia caryophyllata, Eugenia aromatica, Fagara capensis, Humulus lupulus, Hydrastis canadensis, Hypericum perforatum, Jambosa caryophyllus, Juglans regia, Juniperus communis, Kigelia africana, Lavandula officinalis, Lavandula angustifolia, Lygodium circinnatum (N.L.Burm.) Swartz, 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 "GERMICIDE"

Andira araroba, Betula alba, Betula pendula, Carum copticum, Carum ajowan, Citrus mitis, Citrus microcarpa Bge.(by Tanaka), Humulus lupulus, Lavandula officinalis, Lavandula angustifolia, Lithospermum erythrorhizon, Melaleuca alternifolia, Phellodendron amurense, 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

dracunculus, Azadirachta indica, Baptisia tinctoria, Berberis vulgaris, Betula pendula, Betula alba, Boldea fragrans, Boldo boldus, Boswellia thurifera, Brucea javanica, Calendula officinalis, Calluna vulgaris, Cananga odorata, Cannabis sativa, Capsella bursa-pastoris, Capsicum frutescens, capsicum minimum, Carlina acaulis, Carum copticum, Carum ajowan, Carum carvi, Caryophyllus aromaticus, Centaurium erythraea, Centaurium vulgare, Centella asiatica, Cetraria islandica, Chelidonium majus, Chlorophora excelsa, Cinchona succirubra, Cinnamomum cassia, Cinnamomum camphora, Cinnamonium zeylanicum, Citrus bigaradia, Citrus microcarpa Bge.(by Tanaka), Citrus mitis, Citrus bergamia, Citrus sinensis, Citrus limonum, Citrus medica, Cnicus benedictus, Commiphora myrrha, Commiphora molmol, Copaifera officinalis, Copaifera multijuga, Copaifera guyanensis, Copaifera reticulata, Corydalis cava, Corydalis ambigua Cham. et Schlect., Crithmum maritimum, Cupresses sempervirens, Curcuma amada, Curcuma amada, Cymbopogon citratus, Daucus carota, Diospyros mespiliformis, Echinacea angustifolia, Elettaria cardamomum, Elymus repens, Epigaea repens, Erythraea centaurium, Eucalyptus globulus, Eucryphia lucida, Eugenia caryophyllata, Eugenia aromatica, Eupatorium perfoliatum, Fagus sylvatica, Filipendula ulmaria, Foeniculum vulgare, Fragaria vesca, Galium verum, Gaultheria procumbens, Gentiana lutea, Geranium maculatum, Gerardia pedicularis, Geum urbanum, Gleditschia triacanthos, Gnaphalium stoeches, Gnaphalium citrinum, Gnaphalium dioicum, Gnaphalium polycephalum, Gnaphalium arenarium, Gratiola officinalis, Hamamelis virginiana, Hedeome pulegioides, Hedera helix, Heliotropium europaeum, Hieracium pilosella, Houlttuynia cordata, Houyttuyniae cordata, Humulus lupulus, Hydrastis canadensis, Hydrocotyle asiatica, Hypericum perforatum, Hyssopus officinalis, Indigofera tinctoria, Inula helenium, Isatis tinctoria, Jambosa caryophyllus, Juglans regia, Juniperus communis, Lactuca sativa, Lantana camara Linné, Larrea divaricata (DC) Cov., Lavandula angustifolia, Lavandula officinalis, Legusticum levisticum, Levisticum officinale, Lilium candidum, Liquidambar styraciflua, Lonicera caprifolium, Lonicera periclymenum, Lysimachia nummularia, Magnolia glauca, Matricaria officinalis, Melaleuca leucadendron, Melaleuca alternifolia, Melaleuca viridiflora, Melissa officinalis, Mentha piperita, Meum athamanticum, Mimosa tenuiflora, Musa sapientum, Musa paradisiaca, Myroxylon pereirae, Myroxylon balsamum, Myrtus communis, Nabalus serpentaria, Nymphaea alba major aquatica, Nymphaea candida, Nymphaea lotus, Ocimum basilicum, Paeonia officinalis, Pelargonium odorantissimum, Pelargonium graveolens, Pentaglottis sempervirens, Perilla frutescens, Peumus boldus, Phaulopsis barteri, Phellodendron amurense Rupr., Phellodendron amurence, Pilosella officinarum, Pimenta dioica, Pimenta officinalis, Pimpinella anisum, Pinus montana Mill., Pinus pumilio Haenke, Pinus silvestris, Pinus mughus Scop., Pinus mugo Turra, Piper methysticum, Plantago major, Plantago lanceolata, Podalyria tinctoria, Pogostemon patchouli Pellet, Populus tremula, Prunella vulgaris, Psidium guajava, Pulmonaria officinalis, Pyrola minor, Quercus robur, Quercus petraea, Rhus glabra, Rhus aromatica, Rhus cotinus (Cotinus coggyria), Ribes rubrum, Rosa gallica, Rosmarinus officinalis, Rubia tinctorum, Rubia peregrina, Rubus fruticosus, Rubus fruticosus, Rumex acetosa, Sabbatia angularis, Salix vitellina, Salvia sclarea, Salvia multiorrhiza, Salvia officinalis, Sambucus nigra, Sanguinaria canadensis, Santalum album, Sassafras albidum, Satureia hortensis - Summer Savory, Satureia montana - Winter Savory, Saussurea lappa Clarke, Scabiosa arvensis, Scutellaria baiacalensis, Senecio vulgaris, Senecio jacobaea, Serenoa repens, Smilax regelii, Smilax ornata, Solidago virgaurea, Sophora tinctoria, Sphagnum cymbifolium, Spiraea ulmaria, Stachys palustris, Statice caroliniana (limonium), Styrax benzoin, Styrax officinalis, Syzygium aromaticum, Tamarindus indica, Tamariscus narbonensis, Tamarix gallica, Terminalia

avicennioides, *Terminalia glaucescens*, *Terminalia ivorensis*, *Terminalia macroptera*, *Teucrium chamaedrys*, *Teucrium scordium*, *Thymus vulgaris*, *Tilea europaea*, *Trachyspermum ammi*, *Trifolium pratense*, *Trillium grandiflorum*, *Trillium erectum*, *Trillium flavum*, *Trillium pendulum*, *Turnera diffusa*, *Tussilago farfara*, *Vaccinium vitis-idaea*, *Vaccinium myrtillus*, *Verbena officinalis*, *Viola odorata*, *Viola canina*, *Xylopiya aethiopica*, *Zanthoxylum armatum DC.*, *Zanthoxylum armatum DC.*, *Zea mays*.

Appendix VIII

SEARCH ON "ANTIBIOTIC"

Abies cilicia, *Achillea millefolium*, *Acorus calamus*, *Agrimonia eupatoria*, *Agropyron repens*, *Alkanna tinctoria*, *Allium sativum*, *Allium cepa*, *Aloe barbadensis*, *Aloysia triphylla*, *Ananas sativus*, *Ananas comosus*, *Arbutus unedo*, *Arctium lappa*, *Armeria maritima*, *Armoracia rusticana*, *Artemisia tridentata Nutt*, *Calophyllum inophyllum*, *Carlina acaulis*, *Cassia tora*, *Cassia alata*, *Cassia occidentalis*, *Cassia nigricans Vahl ex D.C.*, *Cassia absus*, *Centella asiatica*, *Cera alba*, *Cetraria islandica*, *Cetraria islandica*, *Citrus sinensis*, *Citrus bigaradia*, *Cnicus benedictus*, *Commiphora molmol*, *Commiphora myrrha*, *Curcuma amada*, *Drosera anglica*, *Echinacea angustifolia*, *Elymus repens*, *Evernia purpuracea*, *Ginkgo biloba*, *Gramen caninum vulgatius*, *Hepatica americana*, *Hieracium pilosella*, *Humulus lupulus*, *Hydrastis canadensis*, *Hydrocotyle asiatica*, *Hypericum perforatum*, *Lepidium sativum*, *Lippia citriodora*, *Lupinus sativus*, *Lycopersicon esculentum*, *Lythrum salicaria*, *Mangifera indica*, *Melilotus officinalis*, *Mimosa tenuiflora*, *Musa sapientum*, *Musa paradisiaca*, *Nigella sativa*, *Pentaglottis sempervirens*, *Pilosella officinarum*, *Pinus silvestris*, *Plumbago zeylanica*, *Plumbago europaea*, *Polytrichum spp*, *Prunella vulgaris*, *Raphanus sativus*, *Salix vitellina*, *Santalum album*, *Solanum esculentum*, *Solanum lycopersicum*, *Terminalia avicennioides*, *Terminalia ivorensis*, *Terminalia glaucescens*, *Terminalia macroptera*, *Trichodesma zeylanicum*, *Triticum repens*, *Tropaeolum majus*, *Usnea barbarta*, *Viola odorata*, *Viola canina*.

Appendix IX

SEARCH ON "ANTIMICROBIAL"

Adropogon citratus, *Alkanna tinctoria*, *Allium sativum*, *Aloe barbadensis*, *Aniba rosaeodora Ducke*, *Anthemis nobilis*, *Arctium lappa*, *Arctostaphylos uva-ursi*, *Argemone mexicana*, *Arnica montana*, *Artemisia tridentata Nutt*, *Azadirachta indica*, *Baptisia tinctoria*, *Betula alba*, *Betula pendula*, *Calamintha officinalis*, *Calluna vulgaris*, *Carum carvi*, *Carum petroselinum*, *Cassia nigricans Vahl ex D.C.*, *Cassia alata*, *Cassia absus*, *Cassia occidentalis*, *Cassia tora*, *Caulophyllum thalictroides (L) Michx.*, *Cetraria islandica*, *Cimicifuga racemosa*, *Cinnamomum cassia*, *Cinnamomum zeylanicum*, *Cistus villosus*, *Citrus racemosa*, *Citrus medica*, *Citrus limonum*, *Citrus decumana*, *Citrus paradisi*, *Commiphora myrrha*, *Commiphora molmol*, *Croton spp.*, *Cryptolepis obtusa N.E.Brown*, *Cryptolepis sanguinolenta Schltr.*, *Cumin cyminum*, *Cymbopogon citratus*, *Daucus carota*, *Echinacea angustifolia*, *Eucalyptus globulus*, *Glycyrrhiza glabra*, *Gnaphalium stoeches*, *Gnaphalium polycephalum*, *Gnaphalium citrinum*, *Gnaphalium dioicum*, *Gnaphalium arenarium*, *Guiera senegalensis*, *Houyttuyniae cordata*, *Humulus lupulus*, *Hydrastis canadensis*, *Indigofera tinctoria*, *Juglans regia*, *Juniperus communis*, *Lapacho morado*, *Lapacho colorado*, *Larrea tridentata*, *Larrea divaricata (DC) Cov.*, *Lavandula officinalis*, *Lavandula angustifolia*, *Legusticum levisticum*, *Levisticum*

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*.