Review Article

Unleashing Nootkatone: A Comprehensive Exploration of its Chemistry and Applications in Industry

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ABSTRACT

Nootkatone is a chemical constituent of essential oil that can be extracted from many natural and synthetic sources, including grapefruit and Citrus paradise Macfadyen. Citrus grandis, vetiver grass, grapefruit, *Callitropsis nootkatensis*, pummelo, and *Chrysopogon zizanioides* are examples of natural plant sources. There may be more plant sources as well. Nootkatone that is synthesized finds widespread application in the culinary, cosmetic, and medicinal sectors. Recently, nootkatone was shown to be toxic to arthropods, and specifically against ticks. The ability of natural products to kill arthropods at relatively low concentrations represents an alternative to the use of synthetic pesticides for the control of disease vectors. As a result, there may be an opportunity to create novel tick management solutions that tackle important issues related to both urban and rural settings. Nootkatone is a naturally occurring sesquiterpene that is derived from valencene and found in plant matter and citrus oils like orange and grapefruit. It has been shown to have additional bioactivities, such as pesticidal and insecticidal properties. The non-toxic substance is approved for use in food, cosmetics, and pharmaceuticals, and is a new weapon against insect-borne diseases like Lyme disease and malaria, repelling and killing ticks and mosquitoes. It is approved in several countries for use as a flavour in foods, fragrances, drinks, and personal care products. The high cost of nootkatone when extracted from grapefruit is a major concern. Thus, the present review will focus on the natural insecticide nootkatone, its chemistry, and various industrial applications.

Keywords: Nootkatone, Natural Insecticide, Flavours, Pharmaceutical products, Food Additive.

INTRODUCTION

The rising global human population is placing increasing stress on the current means of food production and agricultural land use. There is an environmental need to maximize the efficient use of land allocated to farming and commercial forestry, while simultaneously reducing the impact of modern farming and forestry practices on the surrounding natural environments. It has been estimated that the largest loss of food material is that of planted, sown, or otherwise nurtured crops and plant propagation material that are not fit for harvesting. Significant additional food loss occurs post-harvest due to insects and mould and rotting of food materials before consumption. One specific cause of food material loss is sap-sucking insects. Insects that feed on sap Plant stems, buds, fruit, leaves, shoots, and cell walls should all be punctured. After enzymes are injected to aid in extraction, the contents of the plant cells are drawn out.

Therefore, vector-borne infectious diseases continue to pose a significant public health threat, resulting in human and economic loss in India[1].

Lyme disease, anaplasmosis, and Rocky Mountain spotted fever from tick bites; West Nile, dengue, Zika, and chikungunya from mosquito bites; and plague from flea bites have all seen a sharp rise in prevalence. Malaria and yellow fever are two of the deadliest diseases in tropical nations; mosquitoes also carry the elephantiasis virus. Ticks carry the deadly Crimean-Congo haemorrhagic fever, whereas sandflies spread kala-azar.

The emphasis on pest control operations to reduce transmission of vector-borne infectious diseases in the past includes area-wide applications of synthetic pesticides potentially leading to environmental contamination such as soil and groundwater pollution, adverse effects on non-target species, and resistance in arthropod pests[2,3]. With practically endless research potential, botanical pesticides offer a secure and reliable substitute for synthetic pesticides. To repel phytophagous insects, plants have coevolved with insects and created phytochemicals also referred to as secondary metabolites. In this field of study, chemicals from plants have been found that have been demonstrated to be harmful to arthropod pests. The chrysanthemum blooms are the source of the well-known chemical class known as pyrethrins. Regrettably, when present in excessive amounts, synthetic derivatives have the potential to harm the environment and human health.

As far as toxicity to mammals, it has been noted that the Alaska yellow cedar active compounds and their eremophilane skeletons are found in consumable foods such as citrus products or some other herbs[1].

Sesquiterpenes are a broad class of terpenoids that are mostly present in Citrus plant essential oils. Brazil accounts for 50% of orange (*Citrus sp.*) production, while Florida accounts for 32%. The remaining 18 percent are made up of others, such as Mexico, India, and Spain. A highly prized sesquiterpenoid phytochemical, nootkatone has a distinctive grapefruit scent and many desirable biological properties that make it useful in aromatics and medications. Nootkatone is found in very small quantities in grapefruit skin and as an ingredient of various essential oils such as vetiver, Alaska yellow cedar tree wood, *Cupressus nootkatensis,* and pummelo[4]. Traces of nootkatone were also found in bergamot, lemon, lime, orange, and tangerine oils. It can also be derived from the grapefruit peels, which are a bitter-sweet citrus fruit known as Citrus Paradise in science. Grapefruit is also known by the scientific names *Citrus racemosa* and *Citrus maxima*. One of the world's most sought-after aromatic compounds is nootkatone, a sesquiterpene-ketone. Even though it makes up only 0.1–0.2% of grapefruit oil naturally, it is the most significant component for the flavour, fragrance, and pharmaceutical industries. For every kilogram of grapefruit, around 400,000 kg of nootkatone are extracted. Despite a decrease in grapefruit output over the past fifteen years, there is still a considerable demand for grapefruit flavour.

NOOTKATONE: CHEMICAL STRUCTURE

The IUPAC name of (+)-nootkatone is (4a,5-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-7-keto-3-isopropenylnaphthalene) as shown in Figure 1 with chemical formula: $C_{15}H_{22}O$, Molar mass: 218.340 g·mol^{-1} appearance: Clear or white crystals, impure samples appear as a viscous yellow liquid, melting point 36 °C, Boiling point 170 °C, Density 0.968 g/mL, insoluble in water, very soluble in ethanol, dichloromethane, ethyl acetate, soluble in hexanes[5].

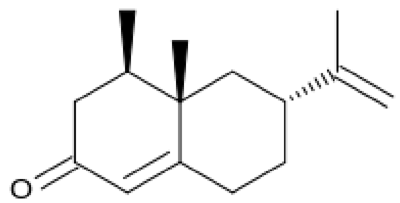

Figure 1: Chemical Structure of Nootkatone

The primary grapefruit scent, nootkatone, is an oxidized byproduct of valencene. It is possible to separate nootkatone from naturally occurring sources like grapefruit, especially grapefruit oil, or Valencia oranges by applying well-established techniques for separating and purifying terpenes. Valencene is oxidized to produce nootkatone. A region-specific hydroxylation process can convert valencene (1,2,3,5,6,7,8,8a-octahydro-7-isopropenyl-l,8a-dimethyl-naphthalene) into 2-hydroxy valencene, which is then further oxidized to generate nootkatone. One can obtain cis and trans-nootkatol by selectively oxidizing valencene at the allylic C2-position. These compounds can then be further oxidized to produce nootkatone. Thus, nootkatone also can be generated by oxidation from valencene using biosynthesis, chemical, or bioconversion methods[6].

EXTRACTION OF GRAPEFRUIT OIL

The bitter-sweet citrus fruit known as grapefruit (also known as citrus paradise; its scientific names are *Citrus racemosa* and *Citrus maxima*) is used to produce grapefruit essential oil. Grapefruit oil is produced from the pericarp (rind) of the ripe fruit, just like all oils made from citrus fruits. To prepare it for cold pressing, the rind is cut off from the pulp. Another method for extracting oil is to soak the rind directly in carrier oils, like coconut or almond, for eight weeks, or eight hours under low heat. Another technique for removing the essential oil from the rind is steam distillation. Since any pesticide placed on the fruit gets concentrated in the oil, the growing method has a direct impact on the quality of grapefruit oil. The best fruit oil for use in cosmetics and medications is that which is taken from organically cultivated fruit.

GRAPEFRUIT ESSENTIAL OIL: CHEMICAL CONSTITUENTS

The gas chromatography-mass spectrometry (GC-MS) showed aldehydes, terpene aldehydes, terpene alcohols, and ketones are major aromatic compounds in most of the citrus essential oils[7]. The most common aromatic chemicals include geraniol, nerol, limonene (citrus), terpinene, α-terpineol (floral), pinene (pine), and linalool (woody). Grapefruit peel and seed oils have more than 200 flavour compounds and nootkatone is the most useful and high-value volatile component of grapefruit peel/seed oils[8].

The most common volatiles in grapefruit essential oil were limonene (96.2%) and myrcene (1.5%), with trace amounts of α-thujene, β-pinene, β-o-cymene, nonanal, iso-geraniol, trans-limonene oxide, α-terpineol, trans-carveol, carvone, citral, geranil acetate, germacrene, and linalool. In cold pressed grapefruit oil had 1,8-cineole (minty), octanal (fresh minty), dodecanal (citrus), trans-4,5-epoxy-(*E*)-2-decenal (green), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (caramel), eugenol (sweet/honey), β-sinensal, and nootkatone as key volatiles[5].

In commercial grapefruit peel oil limonene (93%), myrcene (1.5%), sabinene (0.6%), and α-pinene (0.6%) were found by GC-MS as major aromatic compounds[9]. *Panella* et al.[1] reported essential oils of grapefruit from Pakistan contain 93.9% monoterpenes, and 2.17% sesquiterpenes, among monoterpenes, 86.2% limonene and 6.28% myrcene and α-terpinene was 2.1%.

In the flavour and fragrance market, nootkatone, a sesquiterpene-ketone that is naturally present in trace amounts (0.1–0.2%), is the most sought-after aromatic chemical globally. Nootkatone is so nontoxic to humans that they can even drink it and is approved as a food additive, officially classed as ‘Generally Considered Safe’. It is also a natural ingredient in some foods[4].

INDUSTRIAL APPLICATIONS OF NOOTKATONE

It has demonstrated safety without any sensitization in human non-carcinogen and non-genotoxic. It is approved in all major countries for use as flavour in food formulations, fragrances, drinks, and personal care products. Nootkatone possesses an exceptional aroma that can be detected at a very low threshold level. These aroma chemicals find great applications in the beverage, cosmetic, and pharmaceutical industries[10,11]. Their odour resembles a typical grapefruit and has been reported to exhibit various important therapeutic potentials such as cancer prevention, anti-platelet aggregation, anti-microbial, and anti-inflammation activities; thus embodies an encouraging drug precursor[4,12]. The Center for Disease Control and Prevention (CDC) discovered and created nootkatone, a chemical that kills ticks, mosquitos, and biting insects in addition to repelling pests for several hours. It is now permitted to be used as a novel ingredient in insecticides and insect repellents by the Environmental Protection Agency (EPA). It can kill bugs that are resistant to DDT, pyrethroids, and common insecticides. It has great potential for use as insecticidal soap and, is an impressive repellent for mosquitoes, and ticks even better than synthetics like DEET, picaridin, or IR3535. *Cyperus rotundus* EtOH extract and its component (+)-nootkatone showed antiplatelet effects, suggesting potential therapeutic benefits for preventing platelet-associated cardiovascular diseases[13]. Nootkatone shows low contact toxicity and significant repellence against maize and rice weevils, with potential applications in stored-product insect control[14].

Zhu et al.[15] examined the behaviour of Formosan subterranean termites toward one of the components of vetiver grass oil, the roots of which manufacture insect repellents. The sesquiterpene ketone nootkatone, which was extracted from vetiver oil, was discovered to be both highly poisonous and repulsive to Formosan subterranean termites. Ten µg/g of substrate was the lowest effective concentration that was tested. This is the first documented instance of nootkatone acting as an insect repellent.

According to yet another study, nootkatone is toxic to the tick that spreads disease, Ixodes scapularis, but owing to its volatility, it also causes phytotoxicity to treat plants and has a brief residual activity. To assess a previously used emulsifiable formulation for volatility, plant phytotoxicity, and toxicity to unfed nymphs of *I. scapularis*, the researchers created a lignin-encapsulated nootkatone formulation. The direct method of evaluating nootkatone volatility involved trapping its vapor in a closed system, whereas the indirect method involved measuring the amount of nootkatone residue on treated filter paper following exposure to simulated sunshine (Xenon). Only 15% of the nootkatone applied as the encapsulated formulation and 40% applied as the emulsifiable formulation were recovered by traps after 24 hours in the closed system. The encapsulated formulation maintained 92% of the nootkatone content after a one-hour light exposure, but the emulsifiable formulation only managed to hold onto 26%. Compared to leaves treated with the emulsifiable formulation, cabbage *(Brassica oleracea L.*) treated with the encapsulated formulation showed less necrosis and retained more leaf weight in terms of phytotoxicity. Unlike plants treated with encapsulated nootkatone, which recovered 100%, cabbage and oat (Avena sativa L.) plants only recovered 40% and 40% of the nootkatone in the emulsifiable formulation two hours after administration. Encapsulated nootkatone was shown to be much more harmful to *I. scapularis* nymphs (LC_{50} = 20 ng/cm^2) as compared to the emulsifiable formulation (LC_{50} = 35 ng/cm2). This was determined using a treated vial technique. Thus, the encapsulation of nootkatone improved toxicity for tick control, reduced nootkatone volatility, and reduced plant phytotoxicity[16].

Nootkatone was tested in a recent study against *Aedes aegypti* and *Aedes albopictus*, two mosquito vectors that are significant to medicine. Bottle bioassays were utilized to assess nootkatone's insecticidal potential for both species, whereas biting inhibition and repellency/irritancy bioassays (RIBB) were only employed to test the *A. aegypti* strains. The New Orleans and Vergel strains of *Aegypti*, as well as the ATM-NJ95 and Coatzacoalcos strains of *A. albopictus*, were the insecticide potential of nootkatone that the researchers examined. These strains were selected because Vergel was a verified permethrin-resistant (PERM-R) strain and New Orleans and ATM-NJ95 were permethrin susceptible (PERM-S) strains. The vulnerability of Coatzalcalcos to permethrin was unknown. Insecticides like permethrin are frequently used to manage mosquito populations, and populations of mosquitoes are starting to develop resistance to permethrin. Because permethrin-susceptible and -resistant vectors exist, the researchers aimed to examine the effectiveness of nootkatone (and potential synergy) against these vectors. Both *A. albopictus* strains were at least as susceptible to nootkatone as the New Orleans strain, and bottle bioassays demonstrated that the PERM-R Vergel strain was much less sensitive than the PERM-S *A. aegypti* (New Orleans) strain. Additionally, we demonstrated that New Orleans mosquitoes infected with the Zika virus (ZIKV) were equally vulnerable to nootkatone as were the mock-infected controls. When exposed to nootkatone, the infected Vergel strain showed a considerably lower sensitivity than the ATM-NJ95, Coatzacoalcos, or New Orleans mosquitoes. Overall, our research indicated that nootkatone was unsuccessful against *Aegypti* and *A. albopictus* as an insecticide since it was around 1000× less sensitive than permethrin. RIBB studies, however, showed that nootkatone-treated human subjects' arms prevented *A. aegypti* mosquitoes from searching for their hosts and biting them. RIBB studies concluded that 20% of nootkatone repelled mosquitoes at a rate comparable to commercially available N, N-Diethyl-m-toluamide (DEET; 7%) or picaridin (5%). Nootkatone has the potential to be an efficacious repellent against adult Aedes mosquitoes[17].

It is an additional new weapon to fight against insect-borne illnesses like Lyme disease, and malaria, and repels and kills ticks and mosquitoes. It is even safe to use in the food and fragrance sectors. It is thought to be harmless to humans, other mammals, birds, fish, and bees. In high quantities, the chemical kills mosquitoes, ticks, bedbugs, and fleas in addition to repelling them. In addition, it might work well against other pests including midges, sandflies, lice, and others that can spread deadly illnesses. It lasts for hours and has a pleasant grapefruit-like scent[18].

Nootkatone is also used as a food additive for a food material that is processed food, prepared food, and/or a food ingredient.

Nonetheless, the high cost of nootkatone when extracted from grapefruit is a major concern and natural extraction is proven to be very inefficient, limiting its commercial applications. When made from an extract of oranges known as valencene, the price was marginally lower. Chemical synthesis, which involves the use of ecologically hazardous substances including tert-butyl hydroperoxide, tert-butyl peracetate, and heavy metals as catalysts, is the main method used to meet the high commercial demand for nootkatone[19,20]. With the development of synthetic biology, constructing microbial cell factories represents a promising alternative to produce (+)-nootkatone[10].

There are attempts to locate less expensive nootkatone sources, like byproducts of the citrus and forestry sectors. Furthermore, nootkatol, a distinct type, seems to be a less expensive repellent substitute. Nootkatol is essentially free and is a waste product.

BIOTRANSFORMATION OF SESQUITERPENE IN HIGH-VALUE NOOTKATONE

Nootkatone, a bicyclic conjugated sesquiterpene ketone, is present in both peel-oil-free and peel-rich grapefruit juice. There seems to be a correlation between the relative quantity of nootkatone and the grapefruit oil's flavour strength. It is a high-value ingredient of natural aroma and a new weapon to fight against insect-borne illnesses. It can also be synthesized chemically, but the process is inefficient and usually takes several steps to synthesize with pure chemistry. At every step, there is some loss of the starting material, and the process is uneconomical. An enzyme fermentation process to produce is more cost-effective than using genetically engineered yeast and fermentation technology[10].

Thus, the biotransformation process can be utilized efficiently to convert agri-waste residues (citrus peel) into nootkatone, a natural insect repellent. The process involves the use of cheap sesquiterpene hydrocarbon as a substrate to produce a high-value insecticide using microorganisms such as green algae *Chlorella*, fungi such as *Mucor* species, *Botryosphaeria dothidea*, and *Botryodiplodia theobromae in a simple two-step process*[21].

In a different work by Cankar et al., [22], a *C. nootkatensis* valencene oxidase (CnVO) was found to create trans-nootkatol and (+)-nootkatone by co-expression of putative cytochrome P450 enzymes from *Alaska cedar* in yeast with a valencene synthase. Nootkatone formation (+) was seen at 144 ± 10 µg/L in the yeast culture. CnVO is a member of the CYP706 family of cytochrome P450 oxidases, a novel subfamily.

A valencene synthase gene (CnVS) was discovered in another work through functional evaluation of potential terpene synthase genes obtained from Nootka cypress wood using large-scale EST sequencing. Given the strong correlation between nootkatone content and CnVS expression in various tree tissues, CnVS may be the first specifically designated gene in the nootkatone biosynthesis pathway in *C. nootkatensis*. The protein sequence of the gene bears no resemblance to citrus valencene synthases that are currently known, and it is a member of the gymnosperm-specific TPS-d subfamily of terpene synthases. High robustness under varying pH and temperature regimes is demonstrated by CnVS in vitro, which may be advantageous for application in various host and physiological circumstances. Although it is possible to produce sesquiterpenes biotechnologically, valencene synthase activity must be optimized due to the low productivity of microbial strains expressing citrus valencene synthase. *Saccharomyces cerevisiae's* expression of CnVS suggested the possibility of increased yields. In an optimized *Rhodobacter sphaeroides* strain, expression of CnVS increased valencene yields 14-fold to 352 mg/L, bringing production to levels with industrial potential[23].

Cost Effective Production of Nootkatone from Valencene

The high-value sesquiterpenoid nootkatone is recognized for its impression of grapefruit scent. Low yields occur from its isolation from natural plant sources, and chemical syntheses entail potentially harmful or carcinogenic chemicals. This study presents a biocatalytic method that combines two enzymes in a single pot to synthesize (+)-nootkatone. The sesquiterpene (+)-valencene is first selectively hydroxylated to produce the intermediate alcohol nootkatol by a cytochrome P450 monooxygenase. In the subsequent phase, an alcohol dehydrogenase (ADH) further oxidizes nootkatol to (+) -nootkatone. By carefully choosing a suitable co-substrate for the ADH, which operates in a dual-functional mode, the difficult challenge of identifying an acceptable cofactor regeneration mechanism was resolved. After reaction optimization, involving cosolvent and co-substrate screening, (+)-nootkatone concentrations of up to 360 mg L−1 and a space-time yield of 18 mg L−1 h−1 were achieved[24].

Another approach involves the efficient conversion of commercially available and reasonably priced sesquiterpene (+)-valencene from Valencia orange oil into nootkatone through biotransformations utilizing Noro medium and the following microorganisms: *Fusarium culmorum, Aspergillus cellulosae, Mucor species, Chlorella*, (as shown in Figure 2) and *Botryosphaeria dothidea*. (20 mg 50 ml−1). In addition, *C. fusca* and *C. pyrenoidosa* may readily bio transform 2α-hydroxyvalencene and nootkatol

(2β-hydroxy valencene) in just one day, yielding a high yield (80–90%) of nootkatone. The biotransformation of valencene was by *C. pyrenoidosa* and *C. vulgaris* and soil bacteria also give a good yield[21].

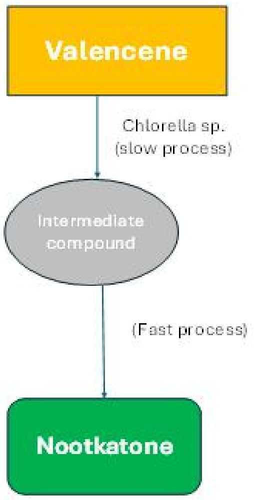

Figure 2: Biotransformation of Valencene by *Chlorella* species

A high yield of 82% was obtained from a fungal strain Mucor sp. that was isolated from the soil, inoculated, and grown statically in Czapek-peptone medium (pH 7.0) at 30 °C for 7 days (20 mg 50 ml−1).

B. theobromae and *B. dothidea* were the biotransformation pathogenic fungi, with 42 and –84%, respectively. It is anticipated that this technique, which uses no heavy metals and is incredibly affordable and clean, will be used in the industrial manufacture of nootkatone.

Bioconversion by micro-algae *Chlorella* is used for hydroxylation and oxidation of organic valencene, converted to the product nootkatone[8].

Recently, engineering nootkatone biosynthesis in *Artemisia annua* using synthetic biology methods provides a green, environmentally friendly, and cost-effective alternative to chemical synthesis. In this study, synthetic biology techniques are used to produce nootkatone in *Artemisia annua*. Nootkatone was generated in the cytosol of engineered *Artemisia annua* by co-expressing valencene synthase (VS) and valencene oxidase (VO), with the yield varying between 0.89 and 8.52 µg/g fresh weight (FW). Additionally, nootkatone ranging from 12.11 to 47.80 µg/g FW was generated by transgenic Artemisia annua co-expressing farnesyl diphosphate synthase (FPS), VS, and VO in plastids. These findings suggested that plastid-engineered nootkatone biosynthesis was preferable to cytosolic biosynthesis. In the meantime, *Artemisia annua*, which produces nootkatone, continued to produce artemisinin. This study developed a green approach for producing nootkatone in *Artemisia annua* with great market potential[25].

Structure-Activity Relationships

The structure and bioactivity potential of monoterpenoids are reflected in their functional group; changes made to this group may result in increased biological activity. The kind and location of the functional groups as well as the molecular structure affect the activities. Therefore, functional groups and optimal structure design are necessary to understand structure–activity relationships. Nootkatone is a keto-sesquiterpene. Its two derivatives, 1,10-dihydro- and tetra-hydro-nootkatone, showed that the repellence of 1,10-dihydro nootkatone and tetra-hydro-nootkatone rose when the hydrogenated double link between carbon position 1 and 10 in nootkatone was removed. Further, the level of unsaturation as revealed in tetra-hydro-nootkatone and 1,10-dihydronootkatone also plays a key role in efficacy[20].

Biotechnological Approaches by Exploitation of P450s

In addition to organic synthetic biology activities, P450 expression in *E. coli* and other bacteria has made it possible to produce very valuable oxidized compounds and convert valencene to nootkatone. The P450 transformed (+)-valencene into (+)-*trans-nootkatone* (70%) and nootkatone (12%) as the major products, and increased nootkatone production to 20% of the overall product[26].

NOOTKATONE: BENEFITS

Unlike the popular traditional insect repellent DEET (N, N-Diethyl-meta-toluamide), nootkatone dries quickly, is non-greasy, and has a very pleasant citrus-grapefruit scent. It is nontoxic and one can drink it. It is approved as a flavour-enhancing food additive, but it is highly toxic to mosquitoes, ticks, and other insects. It is designated as Generally Regarded as Safe (GRAS). Nootkatone is highly effective as an environment-friendly insecticide, and not just against mosquitoes. The main advantage is that it is volatile, does not persist very long in the environment, and is no longer active in soil or groundwater contamination. Further, it has no impact on other non-target insects such as bees and butterflies[27].

MECHANISM OF ACTION

Nootkatone is a unique kind of insecticide that can destroy pests resistant to standard pesticides, pyrethroids, and DDT. It has great potential for use as insecticidal soap, an impressive repellent for mosquitoes, ticks even better than synthetics like DEET, picaridin, or IR3535. Unlike citronella, peppermint oil, and lemongrass oil-based repellents it does not lose its potency even after several hours and lasts long. In many areas, mosquitoes have developed resistance to pyrethrin-based insecticides which are synthetic versions of phytochemicals found in chrysanthemum flowers. In arthropods that are sensitive, nootkatone stimulates the α-Adrenergic Type 1 Octopamine Receptor (PaOA1), resulting in lethal spasms. Mosquitoes are not resistant to it since it functions quite differently from other insecticides, which is a significant issue with most other insecticides on the market. By preventing the neurotransmitter octopamine from binding to receptors on mosquito nerve cells, it kills mosquitoes quickly—typically in a few seconds. The insects become hyperactive as a result, vibrating themselves to death. Since humans lack octopamine receptors, nootkatone may be harmless to them. However, it is unknown if nootkatone and adrenaline receptors interact in any way. In humans, adrenaline is the equivalent of octopamine in insects. Thus, nootkatone works through different mechanisms from previous classes of insecticides[28].

Other Traditional Medicinal uses of *Citrus paradisi*

It has long been utilized as cooling food in the Chinese medical system for patients with excessive body heat. Because people in the West believed the fruit to be a diuretic and aid in weight loss, it became a popular breakfast fruit. Citrus fruit peels have been extensively used as a relaxant, insecticidal, and effective against ticks, mosquitos, and bedbugs[27].

Insecticidal and Antifeedant Activity

Grapefruit peel oil exerted toxicity to adult housefly *Musca domestica* and high fumigant activity against cowpea beetles *Callosobruchus maculatus* (Moravvej and Abbar, 2008). The nootkatone displayed toxicity against fleas with LC_{50} at a very low dose 0.0029% and against nymphs of ticks *Rhipicephalus sanguineus, Ixodes scapularis, Dermacentor variabilis*, and *Amblyomma Americanum* [16].

It showed high insect-repellent activity against cockroaches (*Periplaneta americana* and *Periplaneta fuliginosa*), higher than lemon, lime, and orange oils. It has also been found effectives to control the population of *Callosobruchus maculatus*, *Culex pipiens*, and *Musca domestica,* reduction in egg viability, insecticidal against larvae, growth of pupae, adults, reduction in fecundity and most effective repellents against *Sitophilus oryzae. In addition, as compared to other essential oils, it works better as a repellent against the German cockroach Blattella germanica, the American cockroach Periplaneta americana, and the smokey brown cockroach P. fuliginosa.* The LC_{50} and LC_{90} for second instars of yellow fever vector *Aedes aegypti* after exposure for 24 h were 180.460 and 334.629 ppm, respectively[1].

The current study examined the contact toxicity and repellence of nootkatone against two common pest insects of stored products, the rice weevil (*Sitophilus oryzae* (L.)) and the maize weevil (*Sitophilus zeamais* Motschulsky). A no-choice test with treated filter paper was used to assess contact toxicity, and a choice test with treated wheat or corn (for rice weevils) was used to assess repellences. At tested dosages (range from 11.58 $\mu g/cm^2$ to 1158.08 $\mu g/cm^2$) on filter papers, nootkatone demonstrated minimal contact toxicity (ranging from 0 to 51%) against the two weevil species. In choice tests, there were noticeably fewer maize or rice weevils in corn treated with 0.10% or higher and wheat treated with 0.5% or higher nootkatone compared to the solvent-only treated control, suggesting a repellent effect. The repellence percentage ranged between 46.3 and 93.1% against maize weevils and 39.2–67.2% for rice weevils[14].

Vetiver oil and nootkatone show potential as novel pesticides for substrate treatments, with no adverse effects on pea or citrus plant growth rates. Research has demonstrated the insecticidal and herbicidal properties of vetiver oil and nootkatone, one of its constituents. The substances seem like promising options for new insecticides applied to substrates (such as mulch, wood, and soil). When it comes to soil-dwelling organisms, phytotoxicity is an issue for substrate treatment products.

Using treated potting media, the phytotoxic effect of vetiver oil and nootkatone was evaluated on the pea plants (*Pisum sativum* L.; cv, "Kelvedon Wonder") in the laboratory and on citrus trees (*Citrus unshiu Marcovitch*) under field conditions. At different concentrations (20, 500, and 2000 µg/g soil), every tested chemical inhibited the growth of pea plants in terms of plant height, root length, and dry weight. The least growth-inhibiting treatments were those containing nootkatone, vetiver oil, and disodium octaborate tetrahydrate (Tim-bor ®), a registered pesticide with a history of phytotoxicity. At 500 and 2000 µg/g soil, Tim-bor ® had the most harmful and inhibitive effects, resulting in up to 38.9% plant mortality. Using vetiver and nootkatone oil treatments did not result in any pea plant mortality. The growth rates of citrus plants were not adversely affected by the application of 30 and 100 µg/g potting media treatments of vetiver oil and nootkatone, respectively. These results support the possible usage of the two chemicals around buildings and in nurseries for the management of termites, ants, and weeds; or in the nursery industry where quarantines may be in place to prevent the movement of invasive pests[29].

Cosmetic industry

The grapefruit oil *Citrus paradisi* (pink) has significant importance for cosmetologists due to its purported action on cellulite, water retention, and removal of toxins. Strong antibacterial efficacy against *Propionibacterium acnes*, a primary cause of recurrent acne, as well as antifungal activity against *Penicillium* spp. and growth inhibition against *Staphylococcus aureus* and *Staphylococcus epidermidis*, the bacteria that cause pus to form in pimples, have all been demonstrated in vitro investigations. *In vitro,* antioxidant activity as a *potential* free-radical quencher suggests that it could reduce oxidative stress-induced cellular damage and associated disorders[30]. Nootkatone is known to have a variety of physiological properties, including antioxidative and anti-fibrotic effects. Nootkatone, a naturally occurring sesquiterpene, has been shown to exhibit antibacterial activities against various Gram-positive bacteria, including *Staphylococcus aureus, Enterococcus faecalis, Listeria monocytogenes, Corynebacterium diphtheriae*, and

Bacillus cereus. Notably, the antibacterial effect was most noticeable against C. diphtheriae. Nevertheless, no bactericidal efficacy or growth-inhibitory effects were seen when applied to Gram-negative bacteria. Furthermore, a high concentration of nootkatone was found to have bactericidal effect against Gram-positive bacilli. These findings suggested that nootkatone might target certain metabolites or cell wall components to have an antibacterial impact. Moreover, nootkatone was able to prevent *Staphylococcus aureus* from forming biofilms, even at a low dosage of 0.25 mM. Thus, this study demonstrated the antibacterial efficacy of nootkatone against Gram-positive bacteria, indicating that nootkatone could be a potential candidate for the development of new antibacterial agents[31].

Some of these compounds have been reported to be effective antibacterial agents against *Enterococcus faecium* and *Staphylococcus aureus* in yet another investigation, with minimum inhibitory concentrations (MIC) as low as 1.56 µg/mL. The main ingredient has strong bactericidal effects on *S. aureus* persisters. These compounds are nontoxic to human cancer cell lines at 10 µm concentration[32].

In yet another study successfully produced (+)-nootkatone in *Saccharomyces cerevisiae* by overexpressing (+)-valencene synthase CnVS and various mevalonate pathway engineering strategies, demonstrating a potential sustainable biotechnological approach for industrial demand. By overexpressing the (+)-valencene synthase CnVS, cytochrome P450 monooxygenase HPO, and SDR family dehydrogenases in conjunction with MVA pathway engineering, the researchers were able to successfully construct the (+)-nootkatone biosynthesis pathway in S. cerevisiae, offering a strong foundation for the whole-cell production of (+)-nootkatone. The two effective SDR family dehydrogenases tested in this study will serve as valuable enzymatic tools in further optimizing (+)-nootkatone production[33].

Anticancer activity

Research conducted on cell lines in vitro demonstrated that grapefruit oil's nootkatone has a lethal effect on malignant cells. It has also been demonstrated that nootkatone influences AMPK (AMP kinase), an enzyme involved in energy utilization, which in turn affects the burning of extra fat. A study on squamous cell carcinoma patients showed that a blend of grapefruit, tea tree, and eucalyptus oils along with a Betadine swab resulted in a complete recovery from the necrotizing malodorous wounds[34].

Nootkatone, a bioactive compound in *Alpinia oxyphylla*, exhibits anti-tumorigenic activity in colorectal cancer cells by exhibiting antiproliferative and pro-apoptotic properties. It is well known that *Alpinia oxyphylla* extract has a variety of pharmacological properties. It is uncertain, therefore, how *A. oxyphylla* and the bioactive substance nootkatone contribute to colorectal cancer on a molecular level. In colorectal cancer cells, *A. oxyphylla* extract and nootkatone both show antiproliferative action. In colorectal cancer cells, *A. oxyphylla* demonstrated antioxidant action, most likely through HO-1 induction. Moreover, in the presence of *A. oxyphylla*, the pro-apoptotic protein NAG-1 and the cell-proliferative protein cyclin D1 showed increased and decreased expression, respectively. The administration of nootkatone was found to reduce the formation of spheroid and colony formation when tested for anticancer efficacy. Nootkatone also caused a decrease in cyclin D1 expression and an increase in NAG-1 expression. Nootkatone lowers cyclin D1 through a mechanism involving modulation of protein levels, whereas it enhances the transcriptional level of NAG-1 expression. Apart from its ability to bind to PPARγ, nootkatone also causes a rise in EGR-1 expression, which in turn leads to an increase in NAG-1 promoter activity. Thus, the findings suggest that nootkatone is an anti-tumorigenic compound harbouring antiproliferative and pro-apoptotic activity[35].

Nootkatone was found to impair glucose metabolism and decrease stemness in human breast cancer stem cells in a different study, indicating that it may be a useful therapeutic target for the treatment of breast cancer. The unique metabolic machinery that breast cancer stem cells (BCSCs) exert has a significant impact on radiation and multidrug resistance. Consequently, investigating the processes underlying BCSC energy use may enhance the efficacy of treatment plans intended to eradicate them. This work was carried out to elucidate the mechanisms governing glucose metabolism and the role of nootkatone, a bioactive component of grapefruit, in controlling stemness properties and glucose metabolism in human breast cancer MCF-7 stem cells (MCF-7SCs).

Reverse transcription-quantitative polymerase chain reaction, in silico docking experiments, mammosphere generation, wound healing, invasion assay, transcriptome analysis, seahorse XF analysis, MTT assay, Western blotting, and in vivo investigations were all carried out. Compared to parental MCF-7 cells, MCF-7SCs displayed a higher capacity for tumorigenesis and a unique gene profile with an enrichment of genes related to stemness and glycolysis signalling pathways. These findings suggest that MCF-7SCs obtain their energy from glycolysis rather than oxidative phosphorylation (OXPHOS). Nootkatone decreased the stemness features of MCF-7SCs and impeded glucose metabolism by activating AMPK. The investigation showed that nootkatone is effectively linked to the AMPK active site through in-silico docking. Therefore, this study indicates that the regulation of glucose metabolism through AMPK activation could be an attractive target for BCSCs[36].

Nootkatone, an AMPK activator derived from grapefruit, effectively inhibits non-small-cell lung cancer cell growth and sensitizes them to adriamycin, a promising anti-cancer drug. Both in vitro and in vivo, nootkatone can make ADR-resistant A549/ADR cells more susceptible to ADR. Metformin, on the other hand, failed to show any synergistic effect with ADR in A549/ADR cells[37].

Medicinal Use

Numerous pharmacological actions, including anti-inflammatory, anticancer, antibacterial, hepatoprotective, neuroprotective, and cardioprotective ones, have been described. Although preclinical studies in experimental animal models suggest that nootkatone has therapeutic potential, it is further warranted to evaluate its toxicity and pharmacokinetic parameters before being applied to humans[38]. A wide spectrum of licensed medical professionals as well as self-employed individuals utilize grapefruit essential oil medicinally. Among its many therapeutic qualities are antiseptic, antibacterial, antioxidant, and immune-stimulating effects. It has antiviral and antimicrobial properties[21]. Nootkatone, the most important and expensive aromatic of grapefruit, decreases the somatic fat ratio, and thus its demand is increasing in the cosmetic and fibre sectors[21].

A recent study used an obstructive nephropathy (unilateral ureteral obstructive; UUO) mouse model to examine whether nootkatone medication slowed the progression of chronic kidney disease (CKD) and elucidated its underlying processes. According to the findings, nootkatone therapy considerably reduced collagen deposition, fibrotic marker protein expression, and prevented pathological alterations. In the kidneys of UUO mice, nootkatone may also lessen damage brought on by oxidative stress, inflammatory cell infiltration, and renal cell apoptosis. These findings showed that, in a UUO mice model, nootkatone protected the advancement of CKD for the first time. It may serve as a potential therapeutic candidate for CKD intervention[39].

Aromatherapy

Nootkatone, a natural compound derived from citrus fruits, has transcended its conventional role as a mere fragrance component to become a significant player in the realm of aromatherapy. This aromatic compound, with its distinctive and invigorating citrus scent, offers a multifaceted contribution to the therapeutic landscape, enhancing emotional well-being and mental health. When used in aromatherapy, this oil is frequently used to lower stress, help with anxiety and depression reduction, and improve general energy, focus, and emotional well-being. Its olfactory stimulus activates metabolic pathways that break down fat, raise blood pressure, and cause people to eat less. This makes the oil an attractive option for those who wish to lose weight without strenuous exercise[34]. Inhaling its aroma may induce a state of calmness and tranquillity, promoting relaxation and potentially aiding in sleep. Incorporating nootkatone into nighttime aromatherapy routines can create a serene atmosphere conducive to restful sleep, offering a natural alternative for those seeking relaxation without the use of synthetic sleep aids. At its core, aromatherapy is a holistic practice that leverages the power of scents to influence mood, emotions, and overall mental states. Nootkatone, with its uplifting and refreshing fragrance, serves as a valuable tool in this practice. The inhalation of nootkatone-laden aromas stimulates the olfactory system, which is intricately connected to the brain's limbic system, the seat of emotions and memories. Furthermore, nootkatone contributes to mental clarity and focus. The crisp and bright fragrance has the potential to enhance cognitive function, helping individuals concentrate and sharpen their mental acuity. This aspect of nootkatone in aromatherapy makes it suitable for use in workspaces, study environments, or any setting where mental alertness is desired. A notable aspect of nootkatone's role in aromatherapy is its versatility. It blends seamlessly with other essential oils, allowing for the creation of customized aromatic blends tailored to individual preferences and therapeutic needs. Whether combined with calming lavender, grounding cedarwood, or other complementary scents, nootkatone provides aromatherapists and enthusiasts alike with a creative palette for crafting personalized and effective aromatic experiences.

Food and Beverages

Grapefruit oil has a very distinct aroma and flavour and is often used within the food and beverage industry as a very popular flavouring agent[30]. This is corroborated by a 1967 study in which the flavouring effect of nootkatone, a sesquiterpene ketone that had previously been identified as the main flavouring ingredient in grapefruit oil, was examined in grapefruit juice that had been reconstituted from foam-mat-dried crystals. Unexpected challenges were faced in getting the nootkatone to disperse uniformly. Once a workable technique was created, nootkatone-containing juice samples were compared to controls to determine flavour thresholds and preferences. In water, nootkatone was found at 1 ppm, while in 10.5'Brix reconstituted grapefruit juice, it was found at 6 ppm. The threshold values in the samples with and without "locked-in" oil were identical. Four samples were used to assess the effects of flavour: 1) Nootkatone extracted from grapefruit oil; 2) Nootkatone synthesized from valencene and repeatedly refined by GLC; 3) Synthetic nootkatone regularly purified by GLC; and 4) Nootkatone in crystallized form. Everybody shared the same threshold. Some judges found that nootkatone levels higher than 7 ppm were extremely harsh. For most judges however, at slightly above a threshold level (about 6—7 ppm) the flavour and aroma of grapefruit juice were enhanced by the presence of nootkatone[40].

Health and Beauty

Nootkatone, a naturally occurring compound found in citrus fruits, plays a significant role in promoting both health and beauty. Known for its antioxidant properties, nootkatone helps protect the body and skin from oxidative stress caused by free radicals. This defense mechanism aids in preventing premature aging, reducing the appearance of fine lines and wrinkles, and contributing to overall skin health. Furthermore, nootkatone exhibits anti-inflammatory characteristics, making it beneficial for maintaining clear and radiant skin. It has the potential to soothe irritation and redness, providing relief to individuals with sensitive or problematic skin conditions. Nootkatone's antimicrobial properties also contribute to its role in skincare, as it may help combat certain bacteria that can lead to skin issues. This aspect makes it a promising natural ingredient for maintaining a healthy complexion. Beyond its impact on the skin, nootkatone's invigorating citrus aroma has psychological benefits. The uplifting scent can positively influence mood, reduce stress, and contribute to a sense of well-being. The combination of these health and mood-enhancing effects underscores nootkatone's holistic contribution to both internal well-being and external beauty. The pleasing fragrance and high levels of antioxidants in Grapefruit oil also make it a popular choice within the health and beauty industry as perfume[34].

Nootkatone: Enhancing Bioavailability

Nootkatone (NO) is a volatile sesquiterpenoid flavour that is used in medications, cosmetics, and food products. It also has insect-repellent properties. Its low water solubility and stability limit its applicability; however, this could be remedied by encapsulating it in cyclodextrins (CDs). Phase solubility experiments, isothermal titration calorimetry, nuclear magnetic resonance spectroscopy, and molecular modelling were used in this work to assess the encapsulation of NO by CDs. Using UV-visible spectroscopy, a solid CD/NO inclusion complex was created and its encapsulation efficiency and loading capacity were assessed. Thermogravimetric-differential thermal analysis was used to examine the thermal characteristics, and repeated headspace extraction was used to study release. The production of stable inclusion complexes was demonstrated using formation constants (Kf). The solid complex's NO water solubility, photo- and thermal stability, and guarantee of release from an aqueous solution were all improved. This implies that CDs are prospective carriers to enhance NO characteristics and, as a result, increase their application in agricultural preparations, foods, cosmetics, and medications. Cyclodextrins effectively improve nootkatone's aqueous solubility, and photo- and thermal stability, enabling its wider use in foods, cosmetics, pharmaceuticals, and agrochemicals[41].

CONCLUSION

After considering the aforementioned points, it can be said that nootkatone is a molecule found in essential oils that are used to flavour or smell manufactured goods. It may also be able to manage tick populations in urban areas and ecologically delicate areas in a relatively safe manner. Its distinct qualities, which make it a powerful natural insecticide, have come to light through a thorough analysis of its chemical makeup, which is derived from citrus essential oils. An alternative to using synthetic pesticides to manage disease vectors is the capacity of natural compounds like nootkatone to kill arthropods at comparatively low doses. Therefore, there's a chance to create novel tick repellents that tackle important issues related to both urban and rural settings.

Nootkatone's high value is evident not only in its efficacy in controlling pests but also in its eco-friendly nature, aligning with the growing demand for sustainable alternatives in various industries. The transition from citrus species to the industrial application of nootkatone highlights its adaptability and significance in addressing pest-related challenges. As industries increasingly seek environmentally responsible solutions, nootkatone emerges as a promising candidate, offering a balance between effectiveness and sustainability. The integration of this natural insecticide has the potential to revolutionize pest control practices, providing a safer and more eco-conscious approach in diverse industrial settings.

The key to the future is creating nootkatone encapsulation formulations that can potentially significantly lower losses brought on by exposure to the environment and preserve a sizable nootkatone residual concentration to prolong the chemical's action.

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