ABSTRACT

Metabolism and Pharmacodynamic Effects of Myristicin

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Food chemistry is a branch of science that studies the effects of food and spices on the human body. Nutmeg, a spice most commonly found in desserts and ethnic foods, contains myristicin, a compound that had been studied extensively and found to alter the body's physiology. In this Thesis, the properties, pharmacodynamic effects, metabolism, and potential therapeutic uses of myristicin were investigated and consolidated. Beyond enhancing the flavor of food, myristicin can reduce inflammation by non-selectively inhibiting cyclooxygenase-2 (COX-2), prevent the proliferation of tumor cells by increasing the rate of apoptosis, and decrease oxidative stress by inhibiting the production of radical oxygen species. On the other hand, myristicin can negatively affect the nervous system if an overdose occurs. When a large amount of myristicin is consumed, it can transform into an amphetamine derivative, adopt the conformation of lysergic acid diethylamide (LSD), and cause the user to experience strong hallucinations and symptoms of psychosis. However, despite the adverse side effects, further research should be done into this seemingly innocuous spice to potentially translate these findings into therapeutics for diseases such as cancer, arthritis, and diabetes.

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METABOLISM AND PHARMACODYNAMIC EFFECTS OF MYRISTICIN

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By

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TABLE OF CONTENTS

INTRODUCTION

History of Nutmeg & Common Uses

Eating is a commonplace activity that people partake in every day with little to no regard for how it affects the body. Chilis are julienned and added to dishes to give them a kick. Spices are sprinkled onto food as a flavor enhancer. However, through various laboratory experiments and case studies, researchers have found that spices can go beyond their intended function and affect multiple physiological processes. In this Thesis, the pharmacodynamic effects of myristicin, the active ingredient in nutmeg, will be investigated, and the metabolism and resulting physiological changes produced by the active ingredients in this seemingly innocuous spice will be discussed.

Before the chemical properties of myristicin can be discussed, it is important to consider the history of nutmeg usage to understand the purpose behind this study and why this topic is particularly interesting. Nutmeg is a natural product of the nutmeg tree, *Myristica fragrans* (Figure 1), and is found in the dried seeds of the plant.[1] *Myristica fragrans* originated in the West Indies and was imported by Arabian traders around the first century A.D. Eventually, nutmeg made its way to Europe and captured the attention of the Dutch who monopolized the spice and turned it into a high-priced commodity.[2] Once the monopoly was dissolved and the prices decreased, people began to use it more

[1] Weil, "Nutmeg as a Narcotic."

[2] Weil.

widely for a variety of purposes, such as in cooking or to experiment with its healing properties.

Figure 1: Common Nutmeg (*Myristica Fragrans*)[3]

Nutmeg is most familiar as a finely ground brown powder and has a characteristic aromatic odor and warm, slightly bitter taste. This is due to the volatile oil that comprises up to 40 percent of the nutmeg. The contents of the volatile oil are displayed in Table 1. Volatile oil differs from fixed oil in that volatile oils contain a larger proportion of monoterpenes while fixed oils, whose components are displayed in Table 2, contain a larger proportion of low molecular weight lipids.[4] However, even in the ground form

[3] *Common Nutmeg (Myristica Fragrans)*.

[4] Piras et al., "Extraction and Separation of Volatile and Fixed Oils from Seeds of Myristica Fragrans by Supercritical CO2."

where the concentration is the least, the flavor is still strong which illustrates the potency of this oil.[5]

Name of Chemical Compound	Percentage Found in Volatile Oil
Eugenol and isoeugenol	0.2
d-pinene and d-camphene	80.0
dipentene	8.0
d-linalool, d-borneol, i-terpineol and geraniol	6.0
safrole	0.6
myristicin	4.0
Free myristic acid	0.3

Table 1: Components of Volatile Oil of Nutmeg[6]

[5] Weil, "Nutmeg as a Narcotic."

[6] Weil.

Name of Chemical Compound	Percentage Found in Fixed Oil
Essential oil	12.5
Trimyristin	73.0
Oleic acid	3.0
Linolenic acid	0.5
Formic, acetic, and cerotic acids	Very small amounts
Unsaponifiable constituents	8.5
Resinous	2.0

Table 2: Components of Fixed Oils of Nutmeg [7]

In addition to being added to custards, puddings, eggnog, and other desserts, nutmeg is often used in alternative medicine due to its natural healing properties. For example, some of its uses during the Middle Ages were as a narcotic, a stimulant, and a carminative.[8] More recently, however, the chemical properties of nutmeg have been applied to specific illnesses such as thyroid carcinomas and Crohn's disease. In this context, nutmeg was used to relieve diarrhea by inhibiting the activity of prostaglandins in the gastrointestinal tract as will be discussed in Chapter II: Pharmacodynamic Effects

[7] Weil.

[8] Stein, Greyer, and Hentschel, "Nutmeg (Myristicin) Poisoning — Report on a Fatal Case and a Series of Cases Recorded by a Poison Information Centre."

of Myristicin.[9] Although the spice has been implicated in physiological changes, the mechanism behind this activity has been unknown until recently.

The study that initially sparked interest in this thesis topic was a case study regarding nutmeg overdose. In an article by Brenner, Frank, and Knight, a 25-year-old Caucasian male was believed to have consumed a large quantity of nutmeg.[10] This resulted in symptoms characteristic of psychosis- thought disorder, perseverance of speech, sense of impending death, and auditory hallucinations. However, when the hospitalists attempted to administer large doses of the antipsychotic drug, haloperidol, the patient remained symptomatic and agitated.[11] This suggested that the mechanism by which nutmeg induces psychosis is different from the typical psychosis that is learned in psychology courses.

This study led to further investigation into the use of nutmeg as a narcotic. Because the spice is cheaper than many street drugs, legal, and easily accessible, some people are turning to nutmeg to give them feelings of euphoria. The "high" that results from drug use can be achieved by consuming about 5-15 grams of nutmeg which is equivalent to two teaspoons of ground nutmeg.[12] The effects can be seen, on average, between 0.5 to 8 hours after ingestion and resolve within 1-2 days.[13] During this time, the person can experience a wide range of symptoms from central nervous system (CNS)

[9] Stein, Greyer, and Hentschel.

[10] Brenner, Frank, and Knight, "Chronic Nutmeg Psychosis."

[11] Brenner, Frank, and Knight.

[12] Brenner, Frank, and Knight.

[13] Beckerman and Persaud, "Nutmeg Overdose."

disorder-- anxiety, confusion, dizziness, drowsiness, euphoria, hallucinations, and seizures-- to gastrointestinal distress-- vomiting, excessive thirst, and nausea.[14]

Due to the slow onset and variability in its effects, nutmeg abuse is usually a one-time occurrence and is not as well documented as other drugs used to achieve feelings of euphoria such as marijuana and heroin. Furthermore, there is no known antidote for nutmeg overdose which may discourage abuse.[15] The only treatments available for nutmeg overdose target the symptoms rather than the cause. Standard antiemetics such as prochlorperazine, trimethobenzamide, and metoclopramide can be used to relieve nausea and vomiting, and sedatives such as diazepam and haloperidol can be used to treat hallucinations and delirium.[16] However, these medications have limited efficacy against overdose due to nutmeg's varying, and often unpredictable, physiological effects.

But how does nutmeg exert all these effects on the human body? The answer lies within its main chemical component: myristicin, $C_{11}H_{12}O_3$ (Figure 2).

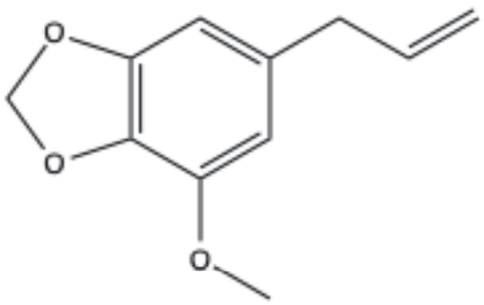


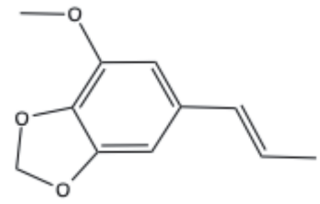
Figure 2: Line-bond structure of Myristicin

[14] Beckerman and Persaud.

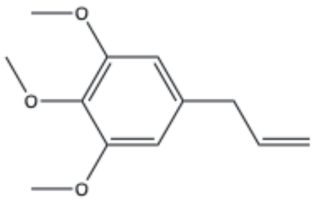
[15] Beckerman and Persaud.

[16] ab. rahman, Fazilah, and Mohd Esah, "Toxicity of Nutmeg (Myristicin)."

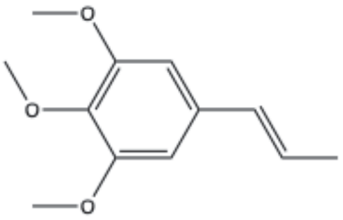
Among other allylbenzene derivatives that exist within the volatile oil of nutmeg- elemicin, safrole, methyleugenol, eugenol, methylisoeugenol, isoeugenol, isoelemicin, and methoxyeugenol- myristicin's effects on the human body have been studied the most extensively, and most of nutmeg's side effects can be attributed to this compound.[17] Some of these other compounds are illustrated in Figure 3.



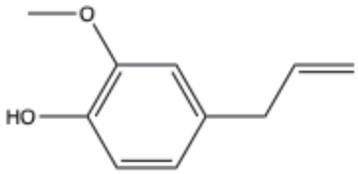
(a) isomyristicin



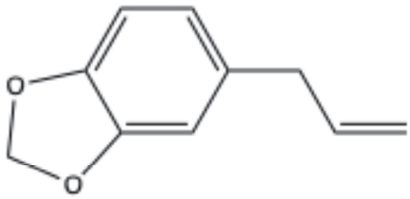
(b) elemicin



(c) isoelemicin



(d) eugenol

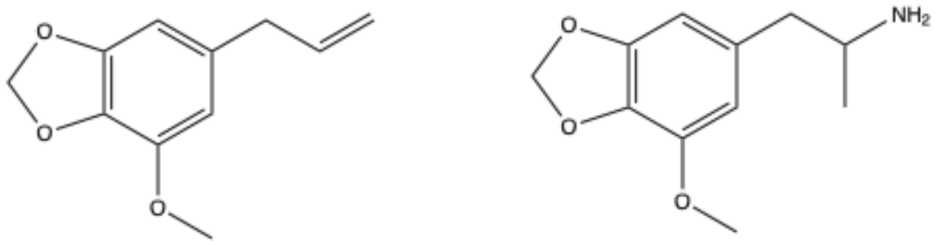


(e) safrole

Figure 3: Compounds related to Myristicin that are found in the volatile oil of nutmeg

[17] Kalbhen, "Nutmeg as a Narcotic. A Contribution to the Chemistry and Pharmacology of Nutmeg (Myristica Fragrans)."

Myristicin comprises a large percentage of nutmeg's volatile oil and exists as a pale-yellow liquid that has a boiling point at 149°C at a pressure of 15 mm[18]. Due to its structural similarities to amphetamine derivatives such as 3-methoxy-4,5-methylenedioxyamphetamine (MMDA), it is believed that myristicin can be metabolized by multiple pathways to yield compounds that act as serotonin agonists and cause LSD-like effects. This will be discussed in the following chapters. The structures of myristicin and MMDA are compared in Figure 4.



(a) (b)

Figure 4: A comparison of structures for (a) myristicin and (b) 3-methoxy-4,5-methylenedioxyamphetamine (MMDA)

[18] Weil, "Nutmeg as a Narcotic."

CHAPTER ONE

Pharmacodynamic Effects of Myristicin

As briefly stated in the Introduction, nutmeg has been used for its natural healing properties for hundreds of years. Because myristicin is a major component of nutmeg essential oil, many of these effects can be attributed to this compound.

Myristicin as an anti-inflammatory agent

Inflammation is a natural process that the body utilizes to repair tissue, fight infections, and prevent further damage. This process can occur both internally and externally and is associated with other medical symptoms such as acute pain sensation, redness, swelling, heat, and scar formation.[1] Depending on the duration of the symptoms, inflammation can be categorized as either acute or chronic. Prostaglandins and nitric oxide (NO) have been implicated as major molecules in the inflammatory response.[2] Prostaglandin H_2 (PGH_2) is derived from arachidonic acid through a reaction catalyzed by cyclooxygenase isoforms and can be converted into one of the four main bioactive types: prostaglandin E_2 (PGE_2), prostacyclin (PGI_2), prostaglandin D_2 (PGD_2), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).[3] The binding of these prostaglandins to the appropriate receptors can activate or mediate cell responses by increasing the concentration of

[1] Schmid-Schönbein, "Analysis of Inflammation."

[2] Salvemini et al., "Regulation of Prostaglandin Production by Nitric Oxide; an in Vivo Analysis."

[3] Ricciotti and FitzGerald, "Prostaglandins and Inflammation."

secondary messengers such as inositol triphosphate (IP_3), cyclic adenosine monophosphate (cAMP), and calcium.[4] NO production by the enzyme nitric oxide synthase (NOS) has been found to attenuate the inflammatory response by increasing the activity of COX and subsequent formation of prostaglandins.[5] While acute inflammation can be beneficial by repairing tissue and resolving infections, chronic inflammation is damaging and is associated with diseases such as ulcerative colitis, cancer, arthritis, and atherosclerosis.[6]

Myristicin can be used as an anti-inflammatory agent because of the compound's ability to suppress the production of multiple inflammatory markers such as calcium, NO, cytokines (IL-6 and IL-10), macrophage inflammatory proteins (MIP)-1α and MIP-1β, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-inducible protein-10, monocyte chemotactic proteins (MCP) MCP-1 and MCP-3, and leukemia inhibitory factor (LIF).[7] Together, these molecules are involved in the chemotaxis of the inflammatory process which means that they are responsible for propagating the chemical signals throughout the affected region and promoting the cellular response.[8] Furthermore, inhibition of cytokines was reported to restrict the migration and growth of neutrophils and macrophages.[9]

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[4] Ricciotti and FitzGerald.

[5] Salvemini et al., "Regulation of Prostaglandin Production by Nitric Oxide; an in Vivo Analysis."

[6] Warsito, "A Review on Chemical Composition, Bioactivity, and Toxicity of Myristica Fragrans Houtt. Essential Oil."

[7] Warsito.

[8] Seneme et al., "Pharmacological and Therapeutic Potential of Myristicin."

[9] Seneme et al.

Myristicin was also reported to non-selectively inhibit an enzyme responsible for prostaglandin production: COX-2.[10] Consequently, myristicin can decrease cellular concentrations of prostaglandins, specifically PGE_2, the main prostaglandin produced by cells of the brain, kidneys, vascular smooth muscle, and platelets.[11]

Antioxidant Properties

Reactive oxygen species (ROS) are free radicals known to cause cell damage. These harmful molecules arise when redox enzyme active sites bind to oxygen rather than their desired substrate leading to the formation of hydrogen peroxide and superoxide via electron transfer.[12] They then can cause damage to all biomolecules including lipids, sugars, proteins, and polynucleotides, and cause oxidative stress.[13] Oxidative stress is involved in the progression of many diseases such as cancer, cardiovascular disease, diabetes, and arthritis.[14] However, there are defense mechanisms against uncontrolled ROS production; the most notable being nonenzymatic molecules- glutathione, vitamin A, C, and E, and compounds present in foods-commonly classified as antioxidants.[15]

Myristicin and other components of nutmeg oil (elemicin, 4-terpineol, safrole, eugenol, trans-sabinene hydrate, β-caryophyllene, and isoeugenol) have been found to

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[10] Seneme et al.

[11] Ricciotti and FitzGerald, "Prostaglandins and Inflammation"; Seneme et al., "Pharmacological and Therapeutic Potential of Myristicin."

[12] Imlay, "Pathways of Oxidative Damage."

[13] Marrocco, Altieri, and Peluso, "Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans."

[14] Marrocco, Altieri, and Peluso.

[15] Marrocco, Altieri, and Peluso.

have antioxidant properties.[16] In studies in which myristicin and eugenol were isolated, both have been found to increase the activity of antioxidant enzymes.[17] Isolated myristicin, *in vivo*, increased the concentration and activity of catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase and decreased the rate of lipid peroxidation, a cellular process that contributes to ROS production.[18] The pharmacological potential of myristicin as an antioxidant has been suggested by additional studies used to confirm whether or not myristicin, rather than other compounds, causes these significant effects. Researchers tested their hypothesis by using three experimental conditions: essential oil of nutmeg containing myristicin, essential oil of nutmeg without myristicin, and pure myristicin. It was found that essential oil without myristicin had minimal sun protection and antioxidant activity. Essential oil with myristicin was reported to have moderate effects, and pure myristicin had the highest sun protective factor and antioxidant activity.[19] Although studies on this property of myristicin are limited, this study implicates myristicin as the main component of nutmeg essential oil responsible for its antioxidant effects.

Anti-proliferative Effects

Essential oil of nutmeg has historically been used as a natural medicine alternative for cancer therapeutics. Although cell proliferation is an essential process for living

[16] Warsito, "A Review on Chemical Composition, Bioactivity, and Toxicity of Myristica Fragrans Houtt. Essential Oil."

[17] Warsito.

[18] Seneme et al., "Pharmacological and Therapeutic Potential of Myristicin."

[19] Seneme et al.

organisms and enables them to repair and maintain homeostatic functions, uncontrolled cell growth is detrimental and can cause cancer. Two of the hallmark features of cancer cells is that they exhibit the ability to bypass cell cycle checkpoints and become resistant to apoptosis.[20] These characteristics have been the target of cancer treatments because if the treatment is able to cause cell death or prevent the cells from multiplying, tumorigenesis will be inhibited.

When isolated myristicin was tested against multiple cancer cell lines such as K-562 (human chronic myeloid leukemia), NCI-H460 (human non-small cell lung adenocarcinoma), and MCF-7 (human breast adenocarcinoma), it demonstrated significant concentration-dependent antiproliferative activity and inhibited cell growth in 50% to 100% of cells at various concentrations.[21] This inhibition of cell proliferation is believed to be a result of myristicin-induced cell apoptosis as a result of various mechanisms including changes in mitochondrial cell membrane potential, cytochrome C release, caspase-3 activation, and fragmentation of DNA.[22] In addition to preventing the expression of specific proteins and promoting apoptosis, myristicin has been shown to downregulate the DNA damage response, so the cancer cells cannot repair their fragmented genes.[23] This will be further discussed in Chapter IV: Myristicin Interactions with Other Metabolic Enzymes and Products.

[20] López-Sáez et al., "Cell Proliferation and Cancer."

[21] Seneme et al., "Pharmacological and Therapeutic Potential of Myristicin."

[22] Seneme et al.

[23] Seneme et al.

Hallucinogenic Activity

Hallucinogens, or psychedelics, are psychoactive substances that alter perception and consciousness, elicit feelings of euphoria, and potentially induce delirium. Compared to other recreational drugs such as marijuana, cocaine, and alcohol, hallucinogens are considered physiologically safe with little to no risk of addiction.[24] Hallucinogens can be either naturally or synthetically derived and include compounds such as lysergic acid diethylamide (LSD), psilocybin, mescaline, N-methyl-D-aspartate (NMDA), and 3,4-methylenedioxymethamphetamine (MDMA).[25] These molecules exert their effects by binding to $(5\text{-HT})_{2A}$ receptors and acting as serotonin agonists.[26]

According to multiple case studies of myristicin overdose, this compound has been found to have hallucinogenic effects. This is due to its ability to be metabolized into amphetamine derivatives and act similarly to LSD. Furthermore, some structural components of the molecule are chemically similar to serotonin and can act as agonists.[27] The metabolic pathway will be further discussed in Chapter III: Myristicin Metabolism.

[24] Nichols, "Hallucinogens."

[25] Nichols.

[26] Nichols.

[27] ab. rahman, Fazilah, and Mohd Esah, "Toxicity of Nutmeg (Myristicin)."

CHAPTER TWO

Myristicin Metabolism

There have been multiple reports of nutmeg abuse due to its hallucinogenic effects if taken at a high enough concentration. After ingesting around 5-15 grams, or approximately two teaspoons of grated nutmeg, the effects begin to appear after six hours. [1] But how do these effects manifest? The answer lies within the metabolism of myristicin.

One of the most studied pathways in the literature is the conversion of myristicin to 3-methoxy-4,5-methylenedioxyamphetamine (MMDA) via a transamination reaction.[2] A proposed mechanism of this transformation is shown below in Figure 5.

[1] Brenner, Frank, and Knight, "Chronic Nutmeg Psychosis."

[2] Kalbhen, "Nutmeg as a Narcotic. A Contribution to the Chemistry and Pharmacology of Nutmeg (Myristica Fragrans)."

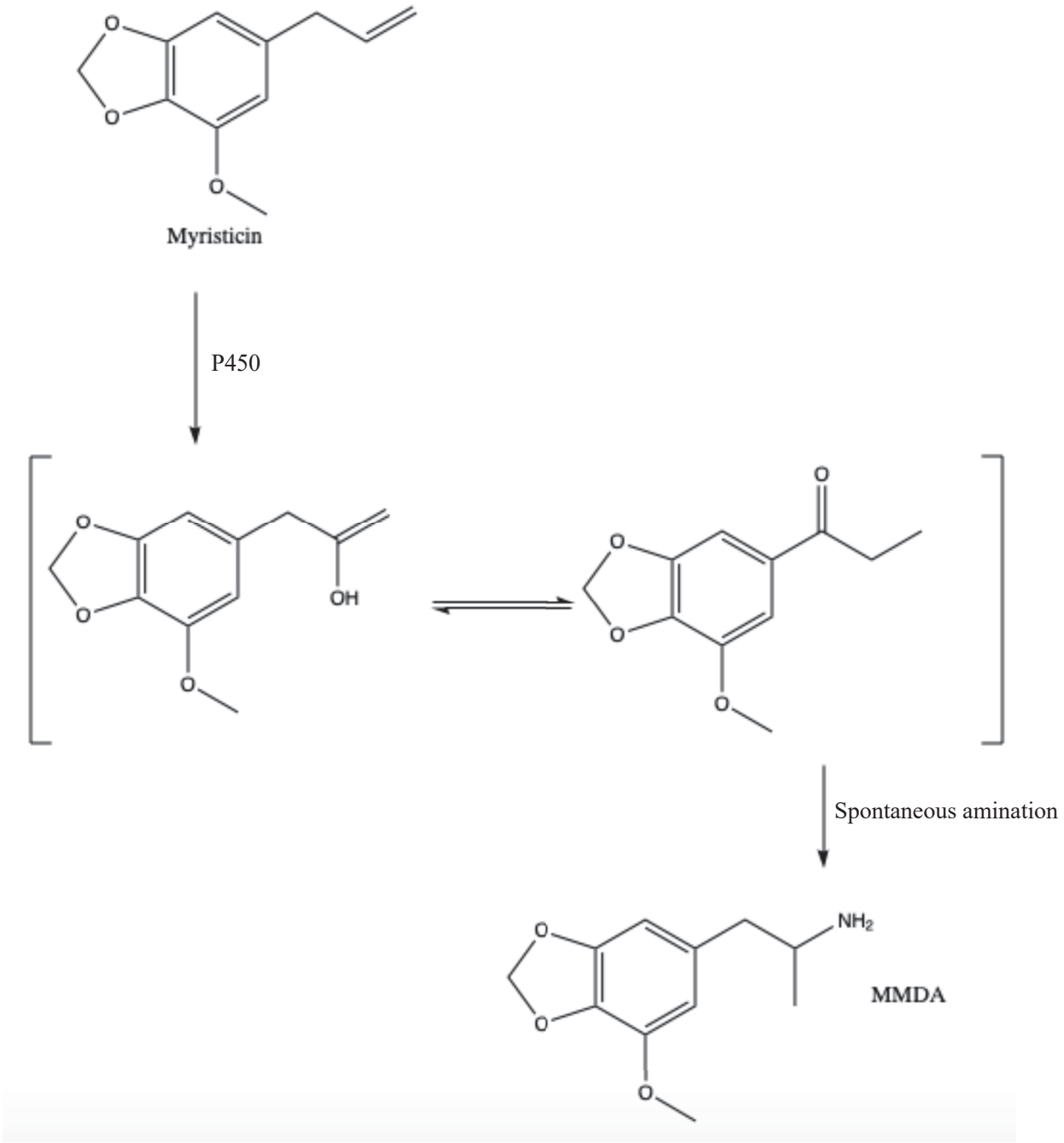

Figure 5: Proposed mechanism of the biotransformation of myristicin into a known amphetamine derivative[3]

[3] Weil, "Nutmeg as a Narcotic."

This biotransformation has also been demonstrated in *in vivo* studies with rats. As mentioned earlier, the conversion of myristicin into the amphetamine derivative, MMDA, involves transamination. However, before this reaction can occur, there must be an oxidation reaction.[4] This idea is supported by a study done by Braun who discovered that when myristicin was incubated in oxygenated rat liver homogenate, the yield of MMDA increased.[5]

Once myristicin is metabolized, it can adopt a configuration similar to lysergic acid diethylamide (LSD), the most potent known hallucinogen. LSD's intense hallucinogenic effects have been attributed to its chemical structure containing an indole skeleton and two fused six-membered rings. [6] Its unique structure may optimize binding to target receptors and enable LSD to have long-lasting psychedelic effects on the user. Although MMDA only contains two rings, the molecule can rearrange itself to create a transient third one. Through intramolecular hydrogen bonding, the amine group can interact with carbon on the ring or the methoxy group to form a structure similar to the one found on the indole skeleton in LSD.[7] This phenomenon is illustrated in Figure 6.

[4] Braun and Kalbhen, "Evidence for the Biogenic Formation of Amphetamine Derivatives from Components of Nutmeg."

[5] Braun and Kalbhen.

[6] Kalbhen, "Nutmeg as a Narcotic. A Contribution to the Chemistry and Pharmacology of Nutmeg (Myristica Fragrans)."

[7] Kalbhen.

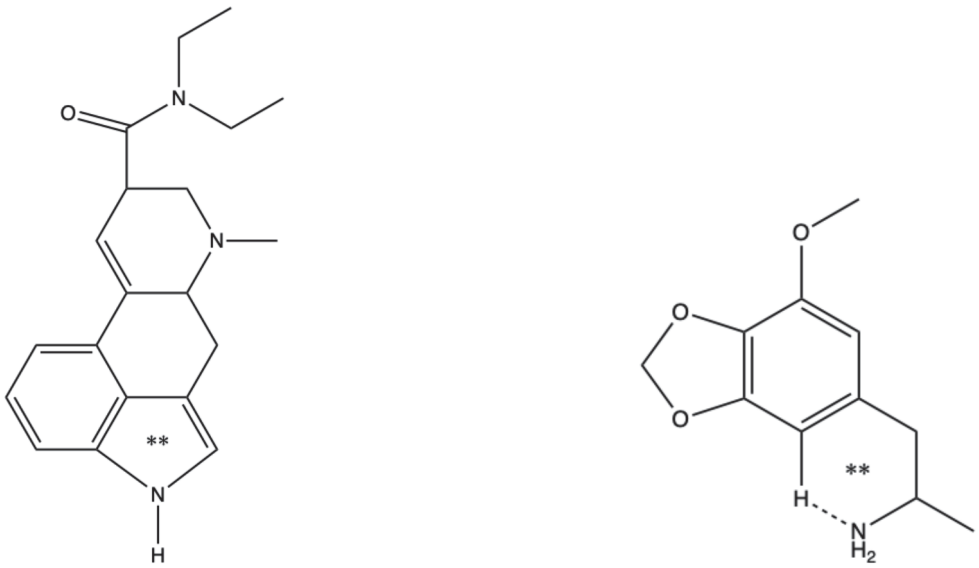

Figure 6: A comparison of the structures of LSD (left) and MMDA (right) with an intramolecular hydrogen bond. ** denotes the analogous rings on both structures

Further support for this mechanism of action comes from studies of 2,3,4-trimethoxyamphetamine, an amphetamine derivative that cannot form hydrogen bonds of this nature.[8] The structure of 2,3,4-trimethoxyamphetamine is shown below in Figure 7.

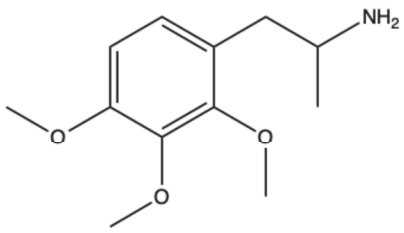

Figure 7: Structure of 2,3,4-trimethoxyamphetamine

[8] Kalbhen.

Due to the positions of the methoxy groups, the ability of the C-2 methoxy group to freely rotate is hindered.[9] When the atoms are unable to adopt a conformation that enables them to form intramolecular hydrogen bonds, no hallucinogenic action is observed.[10] Therefore, it was suggested that MMDA most likely exerts its psychodynamic effects by forming a similar ring structure to LSD and binding to its receptors.

In addition to hallucinogenic effects, myristicin demonstrates toxicity if ingested at high enough concentrations, and this effect has been linked to the way it is metabolized. The metabolism of myristicin has been described to occur in two phases. Phase I is to prepare myristicin for conjugation and involves reactions such as dehydration, hydroxylation, and ring-opening of dioxolane.[11] Phase II involves the conjugation of the modified myristicin to N-Acetylcysteine (NAC) and glucuronic acid.[12] One of the primary reactive metabolites found to cause nutmeg poison is the Myristicin-N-Acetylcysteine (NAC) adduct.[13] Through experiments using cytochrome (CYP) isoforms, researchers have identified CYP1A1 as the main cytochrome responsible for the formation of this toxic metabolite in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH).[14] In addition to creating the myristicin-NAC adduct,

[9] Kalbhen.

[10] Kalbhen.

[11] Zhu et al., "Metabolic Activation of Myristicin and Its Role in Cellular Toxicity."

[12] Zhu et al.

[13] Zhu et al.

[14] Zhu et al.

CYP1A1 is also responsible for the 1'-hydroxylation of myristicin. 1'-hydroxymyristicin demonstrates a lower IC_{50} than myristicin (132.90 ± 1.07 μM compared to 356.4 ± 1.06 μM) which provides further support that metabolic activation contributes to the molecule's toxicity.[15] Once myristicin is converted to 1' -hydroxymyristicin, it can spontaneously bind to NAC to create additional reactive metabolites.[16] The myristicin-NAC adduct can then act as an electrophile and form covalent bonds with biological macromolecules, glutathione, and amino acids such as cysteine.[17] The structure of 1'hydroxymyristicin and the myristicin-NAC adduct is shown below in Figure 8.

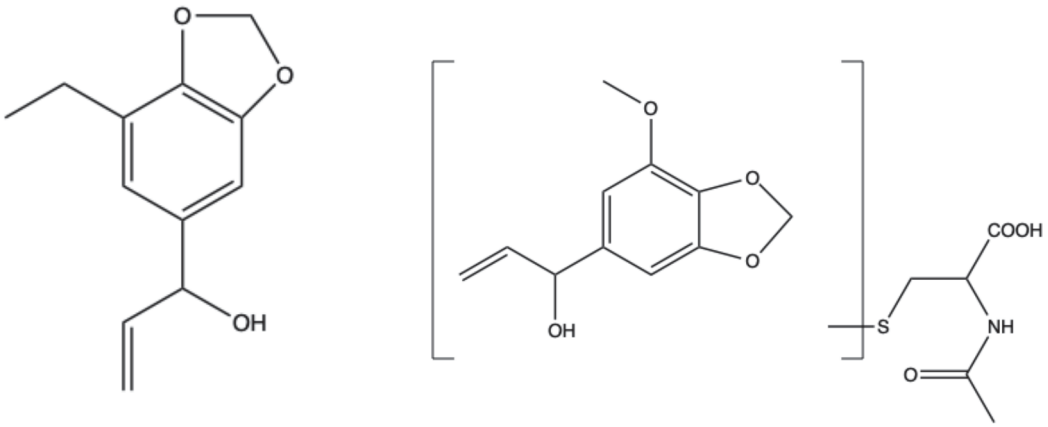

Figure 8: The structure of 1'-hydroxymyristicin (left) and the myristicin-NAC adduct (right)

[15] Zhu et al.

[16] Zhu et al.

[17] Zhu et al.

CHAPTER THREE

Myristicin Interactions with Other Metabolic Enzymes & Genes

As demonstrated in the previous chapters, myristicin can have many pharmacodynamic effects on the human body. As discussed in the previous chapters, ingestion of large amounts of nutmeg (sometimes up to 21 g/person) has negatively affected multiple body systems and caused cardiovascular and neurological symptoms.[1] In addition to these effects, myristicin has been found to induce apoptosis, down-regulate genes involved in the DNA damage response, and alter mitochondrial membrane function.[2] Furthermore, experiments have demonstrated that myristicin can affect cytochrome C activity, most notably cytochrome P450 34A and 2C9.[3]

There have been multiple lines of evidence supporting myristicin's apoptotic effects on cancer cells. In a series of experiments performed by Martins et al., myristicin induced time and dose-dependent apoptosis in human leukemia K562 cells.[4] In the first experiment, the viability of the K562 cells was assessed using an MTT assay. The researchers then dosed the cells with varying concentrations of myristicin ranging from 50 to 1000 μM for 24, 48, and 72 hours. In concentrations greater than 250 μM, cell

[1] Martins et al., "Myristicin from Nutmeg Induces Apoptosis via the Mitochondrial Pathway and down Regulates Genes of the DNA Damage Response Pathways in Human Leukaemia K562 Cells."

[2] Martins et al.

[3] Kimura, Ito, and Hatano, "Effects of Mace and Nutmeg on Human Cytochrome P450 3A4 and 2C9 Activity."

[4] Martins et al., "Myristicin from Nutmeg Induces Apoptosis via the Mitochondrial Pathway and down Regulates Genes of the DNA Damage Response Pathways in Human Leukaemia K562 Cells."

viability was reduced below 50% after 72 hours. When the concentration was doubled to 500 μM, cell viability dropped greater than 20% within both 24- and 48-hour periods.

The MTT assay was followed by a JC-1 assay to elucidate the mechanism through which apoptosis was induced. K562 cells were stained with JC-1, a membrane-permeable cationic fluorochrome, and incubated with varying concentrations of myristicin ranging from 0 to 200 μM. After 20 minutes, researchers found that the red fluorescence decreased in a dose-dependent manner with a 12% decrease for the K562 cells dosed with 50 μM and 39% decrease for cells dosed with 200 μM. This observation suggests that myristicin can cause mitochondrial dysfunction by altering the integrity of the mitochondrial membrane.[5] This was further confirmed by a western blot that revealed cytochrome c release when the cells were incubated with 100 and 250 μM for 6 to 12 hours.

When it was established that myristicin can disrupt the mitochondrial membrane, researchers began to investigate the effects of myristicin on enzymes and genes involved in the apoptotic pathway. When cytochrome c is released from the mitochondria, it binds to apoptotic protease activating factor-1 (Apaf-1) which initiates a cascade of a family of proteases known as caspases.[6] This leads to a formation of a large complex called an apoptosome which recruits and activates an initiator caspase, procaspase-9.[7] Procaspase-9 then induces the downstream processing of caspase-3, one of the main apoptotic enzymes in mammals, and deactivates key structural proteins and essential signaling, homeostatic,

[5] Martins et al.

[6] Shakeri, Kheirollahi, and Davoodi, "Apaf-1."

[7] Shakeri, Kheirollahi, and Davoodi.

and repair enzymes.[8] In an experiment performed by Martins et al., myristicin was found to activate caspase-3 in a dose and time-dependent manner.[9] Caspase-3 activity increased significantly in cells dosed with 250 and 500 μM for 48 and 72 hours. This finding was confirmed with a western blot that measured the cleavage of PARP, a major substrate of caspase- 3. In this experiment, K562 cells were dosed with 100, 250, and 500 μM for 48 and 72 hours.[10] After 48 hours, researchers observed a decrease in the intensity of the largest band and an increase in the intensity of the 85 kDa cleavage product. This further suggests that myristicin activates caspase-3 because myristicin caused the concentration of the PARP cleavage product to increase.

In addition to increasing the activity of the apoptotic enzyme, caspase-3, myristicin has been found to inhibit the activity of cytochromes CYP3A4 and CYP2C9.[11] These cytochromes are predominant in liver microsomes and the intestinal epithelium, and they are responsible for the metabolism of over 50% of clinical drugs including cyclosporin, warfarin, and midazolam.[12] When the activity of the two enzymes was measured in vitro, 10 μM of myristicin inhibited CYP3A4 activity by 40% and CYP2C9 by 2%. Furthermore, myristicin was found to be sensitive to oxidation by CYP34A which

[8] Porter and Jänicke, "Emerging Roles of Caspase-3 in Apoptosis."

[9] Martins et al., "Myristicin from Nutmeg Induces Apoptosis via the Mitochondrial Pathway and down Regulates Genes of the DNA Damage Response Pathways in Human Leukaemia K562 Cells."

[10] Martins et al.

[11] Kimura, Ito, and Hatano, "Effects of Mace and Nutmeg on Human Cytochrome P450 3A4 and 2C9 Activity."

[12] Kimura, Ito, and Hatano.

suggests that competitive inhibition may occur in the CYP3A4-mediated metabolism of pharmaceuticals.[13]

Finally, myristicin has also been found to affect the expression of genes involved in the cell cycle, DNA repair, and apoptosis. When the researchers performed a gene array on K562 cells that were incubated with 100 μM myristicin for 6 hours, they found that out of their 84 genes of interest, 35 of them were significantly downregulated and 17 of them had fold-differences greater than 3.[14] They confirmed these findings with individual TaqMan assays for each gene and found that genes associated with nucleotide excision repair (ERCC1), double-strand break repair (RAD50 and RAD51), DNA damage signaling (ATM), and stress response (GADD45A and GADD45G) were all significantly downregulated.

[13] Kimura, Ito, and Hatano.

[14] Martins et al., "Myristicin from Nutmeg Induces Apoptosis via the Mitochondrial Pathway and down Regulates Genes of the DNA Damage Response Pathways in Human Leukaemia K562 Cells."

CHAPTER FOUR

Modern Uses of Nutmeg in Alternative Medicine

Because of myristicin's various effects on the human body, many people recognize its therapeutic potential. Since ancient times, people in Europe and Indonesia have been using this spice as an herbal remedy for digestive issues, kidney ailments, fevers, headaches, and bronchial problems.[1] Most studies use the essential oil of nutmeg to examine its pharmacodynamic effects, but ground nutmeg, in the correct dosage, can be just as effective. According to a popular handbook on medicinal herbs, a dose of 0.3-1 gram of powdered nutmeg is equivalent to 0.05-0.2 ml of the essential oil.[2]

Perhaps one of the most surprising uses of nutmeg in alternative medicine is to cure male sexual dysfunction. Nutmeg is used in Unani medicine, a form of alternative medicine practiced in Muslim culture in South Asia and modern-day Central Asia, as an aphrodisiac.[3] In a study performed by Tajuddin et al., researchers tested this form of treatment and evaluated the effects of 50% ethanolic extract of nutmeg in rats.[4] When the rates were given a dose of 500 mg/kg for seven days, researchers found a significant increase in sexual activity in the male rats. The rats had significantly increased their

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[1] Parthasarathy, Chempakam, and Zachariah, *Chemistry of Spices*.

[2] Duke and Duke, *Handbook of Medicinal Herbs*.

[3] Tajuddin et al., "An Experimental Study of Sexual Function Improving Effect of Myristica Fragrans Houtt. (Nutmeg)."

[4] Tajuddin et al.

mounting frequency and had greater aggregate penile reflexes with stimulation which suggested that they had greater libido and potency.

Nutmeg is also used to cure insomnia due to myristicin's ability to inhibit monoamine oxidase (MAO), one of the primary enzymes responsible for removing serotonin from neuron synapses.[5] Myristicin, therefore, also acts as an antidepressant by increasing the amount of serotonin acting on the post-synaptic neurons and reducing feelings of depression and anxiety. This is believed to increase brain stimulation because it provides relief from stress which can increase mental capacity and concentration.[6]

Myristicin has also been studied extensively for its potential antimicrobial properties. Microbes are small organisms, such as bacteria and fungi, that are usually associated with illness and disease transmission. These organisms can be dangerous and threaten public health, especially in areas with high population density or inadequate sanitation. Furthermore, with the rise of multidrug-resistant strains of bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), it is important to find alternative ways to reduce microbe growth to limit human contact and transmission.[7]

In a computer assay performed by Swain et al., researchers used computer models to evaluate the inhibitory effects of myristicin against the growth of multi-drug resistant bacterial strains: *Bacillus anthracis*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae,* and *Mycobacterium tuberculosis*.[8] Using molecular

[5] ab. rahman, Fazilah, and Mohd Esah, "Toxicity of Nutmeg (Myristicin)."

[6] "HEALTH AND NUTRITIONAL BENEFITS OF NUT MEG (Mystica Fragrans Houtt)."

[7] Andersen, "Dangerous Microbes."

[8] Swain, Paidesetty, and Padhy, "Development of Antibacterial Conjugates Using Sulfamethoxazole with Monocyclic Terpenes."

docking, researchers determined that myristicin can inhibit dihydropteroate synthase enzyme (DHPS), the enzyme responsible for bacterial folic acid biosynthesis. While myristicin in isolation can prevent the growth of these pathogenic strains of bacteria, its inhibitory effects were stronger when it was conjugated with sulfamethoxazole, an antibiotic.[9]

Furthermore, myristicin can limit the growth of fungi. In a study conducted by Das et al., researchers examined the protective effects of the essential oil of nutmeg (21.29% myristicin) against aflatoxins produced by fungi.[10] They found that the minimum aflatoxin inhibitory concentration of essential oil of nutmeg was 1.5 mg/ml, and the essential oil was effective against a broad spectrum of fungi responsible for food contamination. Upon further investigation of the mechanism by which this inhibitory effect occurs, researchers discovered that the essential oil of nutmeg decreased the ergosterol content of fungal plasma membranes and increased cellular ion leakage. The essential oil of nutmeg also decreased the production of cellular methylglyoxal, an aflatoxin inducer.

In Thai traditional medicine, nutmeg is used as a treatment for malaria, a disease caused by the parasite *Plasmodium falciparum*.[11] Malaria is a disease that is prominent in tropical areas and is characterized by flu-like symptoms such as fever, headaches, nausea,

[9] Swain, Paidesetty, and Padhy.

[10] Das et al., "Assessment of Chemically Characterised Myristica Fragrans Essential Oil against Fungi Contaminating Stored Scented Rice and Its Mode of Action as Novel Aflatoxin Inhibitor."

[11] Thiengsusuk, Chaijaroenkul, and Na-Bangchang, "Antimalarial Activities of Medicinal Plants and Herbal Formulations Used in Thai Traditional Medicine."

vomiting, and diarrhea.[12] In recent years, multidrug-resistant *Plasmodium falciparum* has emerged which caused people in tropical areas to search for alternative treatments. In an experiment performed by Thiengsusuk et al., researchers examined the antimalarial activities of medicinal plants against chloroquine-resistant (K1) and chloroquine-sensitive (3D7) strains of *Plasmodium falciparum*.[13] They found that *Myristica fragrans*, or nutmeg, had median IC_{50} values of 5.4 and 4.6 μg/ml for the K1 and 3D7 variants, respectively. It was classified as a class III antimalarial (moderate to good antimalarial properties) which is defined as having an IC_{50} value between 1.0 μg/ml and 10 μg/ml. After the two strains of *Plasmodium falciparum* were incubated with 50 μg/ml of nutmeg extract for 48 hours, 0.8% and 0.0% of the K1 and 3D7 variants survived, respectively. However, nutmeg was not as potent as the established antimalarial treatments chloroquine, mefloquine, and artesunate which all demonstrated IC_{50} values less than 0.1 μg/ml for both chloroquine-resistant and chloroquine-sensitive *Plasmodium falciparum.* Although nutmeg may not be as effective, it still remains a viable alternative treatment for people in tropical areas that have high rates of multidrug-resistant *Plasmodium falciparum* infection or do not have access to modern medicine.

Finally, one of the nutmeg's most common uses in alternative medicine is for pain management. As discussed in Chapter II: Pharmacodynamic Effects of Myristicin, nutmeg has many anti-inflammatory properties that enable it to treat various illnesses ranging from arthritis to stomach cramps. When applied to affected areas, myristicin can

[12] Bartoloni and Zammarchi, "Clinical Aspects of Uncomplicated and Severe Malaria."

[13] Thiengsusuk, Chaijaroenkul, and Na-Bangchang, "Antimalarial Activities of Medicinal Plants and Herbal Formulations Used in Thai Traditional Medicine."

non-selectively inhibit COX-2, the enzyme responsible for prostaglandin production, and can reduce joint swelling and muscle pain. It has also been used to treat rheumatic fever which is characterized by inflammation of the heart, blood vessels, and joints.[14]

Although the uses of nutmeg in alternative medicine seem promising, there is still much research to be done to ensure that this practice is safe and effective. One of the greatest risks of using herbal remedies is the inability to standardize doses between patients.[15] This is a concern because as mentioned in the previous chapters, myristicin overdose can lead to many undesirable side effects such as vomiting, diarrhea, and hallucinations.[16] In extreme cases, overdose can lead to coma and death. However, with more research, myristicin could potentially be used to treat conditions such as cancer, cardiovascular disease, diabetes, and arthritis. As discussed in Chapter IV: Myristicin Interactions with Other Metabolic Enzymes & Genes, myristicin at high enough doses can induce apoptosis and downregulate genes involved in the damage response which suggests that it could prevent or slow down tumor growth. Furthermore, myristicin's ability to significantly reduce the production of free radicals introduces another avenue for research into its potential to slow the progression of diseases exacerbated by oxidative stress.

[14] "HEALTH AND NUTRITIONAL BENEFITS OF NUT MEG (Mystica Fragrans Houtt)."

[15] Woolf, "Herbal Remedies and Children."

[16] ab. rahman, Fazilah, and Mohd Esah, "Toxicity of Nutmeg (Myristicin)."

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