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Chemical diversity and pharmacological significance of the secondary metabolites of nutmeg (*Myristica fragrans* Houtt.)

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Abstract

Nutmeg is a valued kitchen spice that has been used for centuries all over the world. In addition to its use in flavoring foods and beverages, nutmeg has been used in traditional remedies for stomach and kidney disorders. The antioxidant, antimicrobial and central nervous system effects of nutmeg have also been reported in literature. Nutmeg is a rich source of fixed and essential oil, triterpenes, and various types of phenolic compounds. Many of the secondary metabolites of nutmeg exhibit biological activities that may support its use in traditional medicine. This article provides an overview of the chemistry of secondary metabolites isolated from nutmeg kernel and mace including common methods for analysis of extracts and pure compounds as well as recent approaches towards total synthesis of some of the major constituents. A summary of the most significant pharmacological investigations of potential drug leads isolated from nutmeg and reported in the last decade is also included.

Keywords

Nutmeg; Chromatographic analysis; Biological evaluation; Diarylalkanes; Lignans/neolignans; Phenylpropanoids; Terpenes

Introduction

The ‘western’ history of nutmeg dates back to the 17th century when Nathaniel Courthope, an English spice merchant, succeeded in establishing a route to Run, one of ten volcanic islands in the Banda Sea. These islands are part of the East Indies which are currently located in the Indonesian province of Maluku. During that time, nutmeg was an extremely high valued commodity not only because of its use as an exotic spice but also for its medicinal values (Milton 2000). At present, nutmeg (the seed of *Myristica fragrans* Houtt., family Myristicaceae) still maintains its status as a unique kitchen spice with growing evidence for many of its traditional uses as a natural remedy. The evergreen nutmeg tree can reach 20 m in height and continues to be cultivated in its original location (Indonesian East Indies islands and Srilanka) as well as the West Indies (Caribbean Grenada islands) where

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the tree was introduced in the 19th century (Abourashed and Khan 2010). The lemon-like yellowish fruit contains the seed (nutmeg kernel, Figure 1A) which is enveloped in a reddish net-like spongy tissue known as the aril or arillus (nutmeg mace, Figure 1B). Both kernel and arillus are rich in essential oil that imparts the characteristic aroma and taste to nutmeg as a unique culinary ingredient. Primary metabolites (carbohydrates, lipids/fatty acids and proteins) constitute up to 80% of the weight of dry nutmeg kernel while the remaining weight comprises secondary metabolites of diverse chemical nature. They include essential oils (terpenes and phenylpropanoids) and phenolic compounds (caffeic, ferulic and protocatechuic acids, lignans/neolignans, and diarylalaknes) as the major constituents. Polyphenols and pigments (catechins, epicatechins, falvonoids, and cyanidins) are also present (Table 1). Mace is separately processed from nutmeg and has relatively less fats and carbohydrates.

In folkloric medicine, nutmeg has long been used as a remedy for gastrointestinal problems, such as flatulence, colic, indigestion and diarrhea. The traditional use of nutmeg to treat tumors and infectious diseases, such as parasites and plague, has also been reported. Nutmeg has been used externally to treat skin infections, rheumatism and paralysis (Khan & Abourashed 2010). Other interesting uses for nutmeg include the treatment of psychological disorders (Antonio et al. 2013), and as a cheap substitute for marijuana especially among teenagers, sailors and prison inmates (Weil 1966).

Despite the traditional uses and activities reported for nutmeg, the mechanisms underlying its effects remain unclear and further pharmacological studies are certainly needed to properly assess the therapeutic potential of this natural product. Moreover, reviews and monographs published earlier were very concise in their coverage of the chemistry and pharmacology of nutmeg with more emphasis on the activity of total extracts and essential oils (Abourashed and Khan 2010, Asgarpanah and Kazemivash 2012, Jellin et al. 2003, Latha et al. 2005). Thus, this article will be more focused on pure compounds and will be devoted to reviewing: (i) the diverse chemical structures of secondary metabolites isolated as pure single entities from nutmeg; (ii) recent methods of analysis of major constituents; (iii) representative synthetic approaches to major constituents; (iv) pharmacological investigation of nutmeg extracts and essential oil; and (v) pharmacological effects of pure compounds isolated from nutmeg evaluated in preclinical studies and clinical trials.

Chemistry of major secondary metabolites of nutmeg

Essential oil

The essential oil constitutes up to 16% of nutmeg (w/w) and is rich in monoterpenes (ca. 90%) and phenylpropanoids. A number of recent reports listed between 27–37 components present at various concentrations as determined by GC/MS. In two independent studies by Wahab et al. and Piaru et al., the total number of compounds identified in the essential oil was 37 (Piaru et al. 2012, Wahab et al. 2009). In other studies, Du et al. reported 27 compounds, Muchtaridi and co-workers listed 32 compounds while Piras et al. identified 30 compounds (Du et al. 2014, Muchtaridi et al. 2010, Piras et al. 2012). Based on these most recent reports, the average number of compounds identified in nutmeg essential oil is 34. The monoterpenes -pinene (**1**, 7.4±3.5 %), 4-terpineol (**2**, 16.0±10.6 %), -terpinene (**3**,

5.3±3.3 %), limonene (**4**, 5.9±2.8 %), sabinene (**5**, 16.4±4.8 %), -terpineol (**6**, 2.4±2.2 %), -terpinene (**7**, 4.4±3.7 %), and -pinene (**8**, 5.2±3.0 %); and the phenylpropanoids myristicin (**9**, 12.4±11.7 %), elemicin (**10**, 1.9±1.7 %), methyleugenol (**11**, 3.8±7.2 %), safrole (**12**, 2.6±1.9 %), eugenol (**13**, 6.8±11.4 %), and methylisoeugenol (**14**, 5.7±9.6 %) were the most detected constituents in the five reported samples. Interestingly, isoeugenol the propenyl isomer of **13** or the *O*-demethyl isomer of **14**, was present in trace amounts in only one of the five recent reports on the essential oil of nutmeg (Muchtaridi et al. 2010). Mean concentrations shown between parentheses reflects a wide range of variability (chemical structures are shown in Figure 2).

Lignans and neolignans

Based on the number of identified compounds, lignans and neolignans constitute the most abundant class of secondary metabolites present in nutmeg kernel and mace. The skeleton of a lignan is made of two C6-C3 units (phenylpropanoid dimers) attached at C-2 of both propyl side chains. On the other hand, the dimerization pattern of neolignans involves coupling that is not restricted to both C-2 of the propyl side chains in of the phenylpropanoid monomers (Dewick 1997, Ward 1982, Moss 2000, Solyomvary et al. 2015). Perceiving a phenylpropanoid molecule as having a head (C6 ring) and a tail (C3 unit) a lignan can be loosely identified as a product of tail-to-tail dimerization of two phenylpropanoids while neolignans are formed mainly via head-to-tail coupling, with other possible non-C-2/C-2 couplings. Thus, the basic skeleton of a lignan usually comprises a dibenzyl-substituted tetrahydrofuran, a hexahydrofurofuran or a butane moiety while that of a neolignan may be an ether, a benzofuran, a benzodioxane, or a biphenyl (head-to-head coupling). Due to the abundance of **9**-**14** in nutmeg, all identified lignans and neolignans represent various dimerization patterns between these five phenylpropanoids. A simple schematic of coupling patterns leading to the formation of significant lignan and neolignan skeletons is depicted in Figure 3. It is to be noted that neolignans with hexahydrofuran and benzodioxane skeletons have not been reported in nutmeg.

Numerous lignans have been isolated from nutmeg during routine phytochemical investigation or in the course of performing targeted pharmacological evaluation. At least seven lignans with a tetrahydrofuran 7,7'-epoxylignan nucleus were isolated by Nguyen et al. (compounds **15**-**20**, Figure 4) and by Duan et al. (galbacin, **22**) (Duan et al. 2009, Nguyen et al. 2010). Lignan **21**, probably an isomer of fragransin D1, was identified in nutmeg mace by GC/MS while the 7',9-epoxilignan, **29**, was isolated by Min et al. and a diastereomer of **15** was isolated by Duan et al (Checker et al. 2008, Duan et al. 2009, Min et al. 2011). Lignans with a butane core structure such as macelignan ((8*R*, 8'*S*)-7-(3,4-methylene-dioxyphenyl)-7'-(4-hydroxy-3-methoxyphenyl)-8,8'-dimethylbutane, **23**) and related analogs **24**-**27** were also reported by Min et al. together with the tetralin-type lignan guaiacin, **28**,(Min et al. 2011) which has also been isolated by Nguyen (Nguyen et al. 2010). Lee and coworkers identified related lignans including **30**-**33** (Lee et al. 2009). Lignans are ubiquitous secondary metabolites with special significance in many gymnospermous plant families, such as Oleaceae, Linaceae and Asteraceae (Ward 1995, Umezawa 2003, Schmidt et al. 2010, Szokol-Borsodi et al. 2012, Boldizsar et al. 2010 a & b, Solyomvary et al. 2015). They are also present in other species of genus *Myristica*, such as the dilignan argenteane,

34, isolated from the mace of *M. argentea* (wild or false nutmeg) and the lignan ketone 1-oxo-otobain isolated from *M. simarum* (Calliste et al. 2010, Kuo et al. 1976). It is to be noted that more than one numbering system may be adopted in the nomenclature of lignans as shown in Figure 4 (alternative numbering system shown in parenthesis for compounds **15**-**21**). For the major part of this review the biosynthetically-related numbering system (C1-C9 + C1'-C9') will be adopted for both lignans and neoligans.

Reports of compounds with a neolignan skeleton started to appear in literature in the early 1970s (Isogai et al. 1973). Among the commonly encountered benzofuranoid neolignans are licarins A, B, C & E and their related analogs (**35**-**39**, Figure 5A) which are present in nutmeg as well as other genera such as *Aristolochia*, *Machilus*, *Nectandra*, and others (Aiba et al. 1977, Giang et al. 2006, Leon-Diaz et al. 2010). Additionally, the two benzofuranoid neolignans (7*R*,8*R*)-7,8-dihydro-7-(3,4-dihydroxyphenyl)-3'-methoxy-8-methyl-1'-(*E*-propenyl)benzofuran (**40**) and 3-(4'-allyl-2',6'-dimethoxy-phenoxy)-2-methyl-6-methoxy-2,3-dihydrobenzofuran (**41**) were isolated and reported as new by Duan et al. and by Chiu et al., respectively (Chiu et al. 2016, Duan et al. 2009).

In addition to benzofuranoid neolignans, new 8,4'-oxyneolignans (also called 8.*O*.4'-neolignans) continue to be identified in nutmeg. Cao et al. isolated the five new neolignans myrifralignans A-E (**42**-**46**, Figure 5B) together with **47** (Cao et al. 2015). Related analogs, such as myrislignan (**48**) (Cao et al. 2015), raphidecursinol (**49**) (Cao et al. 2015), surinamensin (**50**) (Francis et al. 2014), *erythro*-(7*S*,8*R*)-D8'-7-acetoxy-3,4,3'5'-tetramethoxy-8-*O*-4'-neolignan (EATN, **51**) (Kang et al. 2013) and others (**52**-**58**) (Cao et al. 2015) have also been isolated from nutmeg.

Diphenylalkanes

In addition to lignans, which can be chemically classified as diphenylbutanes, two types of diphenylalkanes have been identified in nutmeg. The diphenylnonanoid malabaricones A-D were originally isolated *M. malabarica* (wild nutmeg), and, of these malabaricone B and C (**59** & **60**, respectively, Figure 6) are among common constituents later isolated from the kernel and mace of nutmeg as well as the bark of another Myristica species, *M. cinnamomea* (compound **60**) (Chong et al. 2011, Orabi et al. 1991, Purushothaman et al. 1977). Another -terpineol ether of malabaricone C (**61**) was isolated and reported as a new compound by Duan et al (Duan et al. 2009). 1,3-Diphenylpropanes constitute a number of less common diphenylalkanes that were recently reported by Cuong et al. in the course of a bioassay-directed isolation project. Three compounds were isolated and identified as 5-((2*R*,3*S*)-4-(4-hydroxy-3-methoxyphenyl)-3-methylbutan-2-yl)-3-methoxybenzene-1,2-diol (**62**), (*S*)-4-(1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)propyl)benzene-1,3-diol (**63**) and (*S*)-4-(3-(benzo[d][1,3]dioxol-5-yl)-1-hydroxypropyl)benzene-1,3-diol (**64**) (Cuong et al. 2011). It is noteworthy that diarylpropanoids reported by Forrest et al. in 1974 are more appropriately classified as neolignans based on their benzofuranoid and 8,4'-oxyneolignan core structures (Forrest et al. 1974).

Phenylpropanediols, steroids and cyclobutanones

The four phenylpropanoids (**65**-**68**) shown in Figure 7 were reported by Duan et al. and are diol derivatives of isoeugenol, elemicin, myristicin and safrole, respectively (Duan et al. 2009).

The two ubiquitous steroids -sitosterol (**69**, Figure 7) and its 3-glucoside daucosterol (**70**) have been identified in nutmeg (Hou et al. 2012).

Two conflicting reports about the presence of cyclobutanones in nutmeg have been published. 2-Alkylcyclobutanones (2-ACBs) are unique radiolytic products resulting from irradiation of food products to eliminate microbial pathogens (Variyar et al. 2008). By utilizing supercritical fluid extraction coupled with TLC, GC and MS, Variyar et al. demonstrated that 2-decyl- and 2-dodecylcyclobutanone (compounds **71** and **72**, respectively, Figure 7) were present in nonirradiated nutmeg samples (Variyar et al. 2008). More recently, however, Leung et al. disputed earlier findings after applying LC-MS to determine the levels of 2-ACBs in both natural and irradiated nuts including nutmeg. Analytical results of showed that 2-ACBs were only detected in irradiated samples. The authors concluded that 2-ACBs either do not exist in non-irradiated nuts or that they may be present at very low concentrations for reliable detection (Leung et al. 2013).

Analysis of major constituents of nutmeg

As shown in Table 1, primary metabolites such as fixed oils, pigments, starch and protein constitutes more than 70% of nutmeg kernel’s weight. Analysis and fingerprinting of nutmeg secondary metabolites is generally more focused on its volatile oil. Thus, many methods utilizing gas chromatography with or without mass spectrometric detection (GC or GC/MS) have been reported for this purpose in the last decade as discussed under essential oil constituents. On the other hand, relatively few analytical methods have been reported for profiling non-volatile secondary metabolites in nutmeg extracts and preparations. Two recent reports utilized high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) for qualitative analysis of nutmeg samples. Tripathi and Dwivedi utilized normalphase HPTLC for fingerprinting and standardization of nutmeg samples from India (Tripathi and Dwivedi 2015); while Chiu et al. employed reversed-phase HPLC to guide the isolation and identification of thirteen volatile (phenylpropanoids) and non-volatile constituents (neolignans and malabaricones) from nutmeg samples purchased in the United States (Chiu et al. 2016). As for quantitative analysis, the most recent method employed ultra-performance liquid chromatography/mass spectrometry (UPLC/MS) to determine 16 constituents in nutmeg fruit extracts and Indian polyherbal formulations (Pandey et al. 2015). The last validated method for simultaneous determination of multiple constituents was reported by Ehlers et al. more than 15 years ago in which the levels of nine phenylpropanoids were determined in supercritical fluid extracts of nutmeg kernel and mace by reversed-phase HPLC (Ehlers et al. 1998). A GC method was also developed by Dawidowicz and Dybowski for determination of myristicin only as a common constituent of many nutmeg-containing spices. Solid phase extraction of myristicin from pressurized and ultrasonic-assisted liquid extracts was utilized to improve chromatographic performance (Dawidowicz and Dybowski 2012). A summary of

chromatographic conditions and analyzed constituents of the above mentioned methods is shown in Table 2.

Bioanalytical methods for detection and quantitation of individual components of nutmeg and/or their metabolites have also been published in conjunction with biological evaluation and pharmacokinetic profiling of these compounds. Myristicin (**9**) and licarin A (**35**) are the most commonly bioanalyzed single constituents of nutmeg in recent literature. For example, Dawidowicz and Dybowski developed a method for determination of myristicin in human serum to aid in the identification of nutmeg poisoning and intoxication (Dawidowicz and Dybowski 2013). In one study to evaluate the anxiogenic effect, licarin A was quantified in rat cerebral nuclei using HPLC with UV-detection (Zhang et al. 2013). Licarin A bioanalysis was also reported as part of three separate pharmacokinetic studies utilizing HPLC for analysis. In the first two studies, Li and Yang identified nine licarin A metabolites in rat urine and feces and after incubation with liver microsomal fractions during the course of an anti-inflammatory evaluation study (Li and Yang 2011, Li and Yang 2012). Eight phase 1 metabolites resulting from hydroxylation, *O*-demethylation, dehydrogenation and furan ring opening (**73-80**) were obtained in addition to one phase 2 acetylated metabolite (**81**). As shown in Figure 8, metabolites **73-76** were generated *in vivo* while metabolites **76-81** were generated *in vitro*. Metabolite 76 was the only metabolite detected both *in vitro* and *in vivo*. All metabolites were isolated and identified by a combination of solvent extraction, chromatographic and spectroscopic methods. The third study, investigated gastrointestinal absorption of ten neolignans, including licarin A, methoxylicarin A, licarin B, myrislignan and others in the Caco-2 cell permeability assay (Yang et al. 2010). The study showed that transport of the 8,4'-oxyneolignans, such as myrislignan, across Caco-2 monolayers was superior to that of the benzofuranoid-type licarins.

Thin-layer chromatography (TLC) has been utilized for detection of contaminants in nutmeg products as reported by Takahashi who claimed that more than 43% of sixty-seven nutmeg samples available in Japan contained aflatoxins (Takahashi 1993). A more recent method for detection of aflatoxins and ochratoxin A has been reported by Kong et al. whereby HPLC coupled with post-column derivatization and fluorescence detection was employed to determine contamination levels in thirteen nutmeg products purchased in China (Kong et al. 2013).

Approaches towards chemical synthesis of selected active constituents of nutmeg

Total synthesis of natural products is often utilized to support the identification of newly reported secondary metabolites, to supply sufficient material for biological evaluation, structure-activity relationship studies and product development as well as demonstrating the utility of a new chemical transformation and/or reagent. It seems that most promising biologically active secondary metabolites of nutmeg are either lignans/neolignans or diarylnonanoids. Although isolation from crude nutmeg is more commonly utilized to supply purified compounds for biological evaluation, some bioactive lignans/neolignans and

diarylnonanoids have been prepared via total synthesis. Chemical approaches to synthesis of selected members of each class will be highlighted below.

Synthesis of lignans and neolignans

Chemical literature is populated with numerous synthetic pathways to diverse lignan and neolignan skeletons, including two early comprehensive reviews by Ward (Ward 1982, Ward 1995). Dihydroguaiaretic acid (DGA, **30**) is one of nutmeg's butane-type lignans whose *meso*- form was successfully synthesized by Kawaguchi et al. together with many related isomers and analogs (Kawaguchi et al. 2009). A multistep stereoselctive synthesis starting with an anti-Evans's aldol product (**82**) and involving sequential reductive/oxidative cleavages and aldol condensation lead to *meso*-DGA in 5% yield (Figure 9). One general method for synthesis of benzofuran-type neolignans was reported by Engler and Chai (Engler and Chai 1996). In this procedure, racemic licarin B (**37**) was prepared in ~25% overall yield from *trans*-isosafrole (**83**) and a substituted quinone sulfonimide (**84**) as starting materials for the benzofuran nucleus (**85**) followed by multiple steps to attach the propenyl moiety (Figure 10A). Stereoselective synthesis of non-benzofuranoid neolignans has also been achieved. To demonstrate, Zacchino and Badano conducted a two-step sequence starting with a coupling reaction between 1-(3,4,5-trimethoxyphenyl)-2-bromopropan-1-one (**86**) and isoeugenol (**87**) followed by reduction of the ketone product (**88**) with $NaBH^4$ to generate (+)-surinamensin (**50**) in *threo*/*erythro* ratio of 9/1 and 50% overall yield (Figure 10B) (Zacchino and Badano 1985).

More recently, biomimetic oxidative coupling has emerged as one of the most popular approaches towards lignan/neolignan synthesis (Kishimoto et al. 2015, Lindsley et al. 2011). For example, Liu et al. recently reported the synthesis of licarin A (**35**) via silver oxide-catalyzed dimerization of isoeugenol with a 40% yield after reflux in dry toluene/acetone (2:1, v/v) for 48 h at 70–75 °C (Liu et al. 2013). Iodobenzene diacetate is another reagent that was successfully utilized by Juhasz et al. to catalyze the dimerization of isoeugenol to **35** and 3 other neolignans (Juhasz et al. 2000).

Synthesis of diarylnonanoids

The earliest total synthesis of a malabaricone was reported by Parthasarathy and Gupta to confirm chemical structure of malabaricone A (isolated from *M. malabarica* and *M. dactyloids* but not from *M. fragrans*) (Parthasarathy and Gupta 1985). Current total synthetic approaches to various malabaricones, including those present in nutmeg (**59** and **60**), are based on the general scheme reported by Tsuda, Hosoi and co-workers (Hosoi et al. 1999, Tsuda et al. 1991). The general approach is based on multi-step derivatization of a 6-benzyloxyhexylphosphonium salt (phosphorous ylide **89**, Figure 11) at both ends with different aromatic aldehydes and ketones to generate various diarylheptanoids including malabaricone A-D. The overall yield of this scheme is ~31% for **59** and **60** and is shown in figure 10 for **59**.

Pharmacological investigation of nutmeg extracts and essential oil

Despite the myriad of folkloric uses of nutmeg, preclinical and clinical studies supporting such uses are relatively limited. Pharmacological studies have confirmed a few activities of nutmeg extracts, including antidiarrheal, antimicrobial, antioxidant, and different CNS activities. Gorver et al. examined the effects of crude nutmeg suspension, aqueous, as well as petroleum ether extracts in a variety of activities (Grover et al. 2002). The study revealed a significant antidiarrheal effect exerted by both the crude suspension and petroleum ether nutmeg extract. However, only the petroleum ether extract had a significant sedative effect. None of the extracts showed significant cardiovascular effects as measured by blood pressure and ECG changes. On the other hand, a cardioprotective effect against myocardial infarction (MI) has been reported. Abdul Kareem et al. examined the effect of daily administration of aqueous nutmeg extract (100 mg/kg, p.o.) for 30 days on isoproterenol-induced MI in adult male rats (Abdl Kareem et al. 2009). Data collected show that pretreatment with nutmeg extract offered protection against isoproterenol effects on blood glucose, plasma lipids, as well as histological myocardiac changes, suggesting a potential cardiovascular protective role of nutmeg consumption. Very few studies examined the antimicrobial activity of nutmeg extracts. Methanolic extracts possess potent antifungal activity against various plant pathogenic fungi, with three lignans (*erythro*-austrobailignan-6 [macelignan, **23**], *meso*-dihydroguaiaretic acid [**30**] and nectandrin B [**19**]) identified as the primary constituents responsible for the reported antifungal activity (Cho et al. 2007). Similar antimicrobial activity was reported for nutmeg against the pathogenic *Escherichia coli* O157 and O111. The activity proved to be selective against the pathogenic versus the non-pathogenic *E. coli* strains (Takikawa et al. 2002). On the other hand, evaluation of nutmeg ethanolic extract against clinical isolates of *Staphylococcus aureus, E. coli*, and *Streptococcus pyogenes* resulted in lack of antimicrobial activity against all tested bacteria (Sattaponpan and Kondo 2011). Ethyl acetate and ethanolic extracts of the seed, mace, and flesh of nutmeg demonstrated high bactericidal activity against several Gram positive and Gram negative oral pathogens (Shafiei et al. 2012). *In vitro* evaluation of antimalarial activity of 27 herbal extracts and 5 formulations proved nutmeg to be among the identified eight extracts that exhibited potent antimalarial activity with IC_{50} value less than 10 mg/mL. In addition, the activity showed a selectivity index of >10 against multidrug resistant *Plasmodium falciparum* versus human renal epithelial cells (Thiengsusuk et al. 2013). Chemical investigation of nutmeg resulted in the isolation of a wide range of phenolic compounds belonging to the lignan group. With such compounds identified, research efforts intensified at evaluating the antioxidant potential of nutmeg. A study conducted by Assa et al. examined the antioxidant capacity of methanolic extracts of nutmeg mace, seed, and flesh (Assa et al. 2014). Results attributed the highest free radical scavenging antioxidant activity to the seed extract based on the 1,1-diphneyl-2-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assays. Phytochemical evaluation correlated the high antioxidant capacity of the seed extract to its tannin, terpenoid, and flavonoid components. Lack of antioxidant activity of mace was also reported by Yadav and Bhatnagar and was attributed to the relatively low polyphenolic content (Yadav and Bhatnagar 2007). The antioxidant properties of the aqueous extract of nutmeg seem to be responsible for its observed

antimuatgenic and antimitotic actions against the cyclophosphamide-induced carcinogenic effects in the *Allium cepa* test (Akinboro et al. 2011).

The most studied pharmacological activity attributed to nutmeg is its effects on the central nervous system (CNS). As early as the 12th century, nutmeg has been used and known for its CNS activity. Available literature has recognized a variety of nervous system effects of nutmeg and its major constituents. Earlier anecdotes report psychoactive and hallucinogenic properties of nutmeg (Truitt et al. 1961). These reports were the basis of Shulgin's hypothesis that attributed nutmeg's psychoactivity to metabolic conversion of its main phenylpropanoid constituents to amphetamine-like metabolites (Shulgin 1966). So far, the hypothesis has not been experimentally supported. Inconsistent animal findings and lack of detection of the amphetamine-like metabolites in biological fluids of nutmeg abusers led to reevaluation of the validity of the hypothesis (Beyer et al. 2006, Braun 1973). Further experimental data have ascribed several additional nervous system effects to nutmeg. Hayfaa et al. reported analgesic activity of acidulated ethanolic extract of nutmeg (1 g/kg dose) in acetic acid-induced writhing animal model (Hayfaa et al. 2013) in support of earlier reports of the analgesic activity of the *n*-hexane nutmeg extract (Grover et al. 2002, Sonavane et al. 2001). Neurobehavioral effects exerted by nutmeg have been documented in various animal models, with numerous activities reported. Sonavane et al. reported an anxiogenic activity exerted by the *n*-hexane extract of nutmeg, at doses of 10 and 30 mg/kg, i.p., as well by trimyristin (10, 30, and 100 mg/kg, i.p.) (Sonavane et al. 2002). On the other hand, Ayurvedic literature reports the use of aqueous nutmeg extract as an anxiolytic agent (Sharma 2001). Such claim has not been substantiated by experimental dependent anxiolytic activity of aqueous nutmeg extract in the open field test experimental model. Similar to reported results for its effect on anxiety, conflicting data have been documented for nutmeg's (and its components) effect on depression. Dhingra et al. and Moinuddin et al. reported antidepressant activity of nutmeg extracts in both models of behavioral despair as well in reserpine reversal test paradigms, respectively (Dhingra et al. 2006, Moinuddin et al. 2012). The studies also suggested the involvement of adrenergic, serotonergic, and dopaminergic systems in the observed antidepressant effect, since it was inhibited by $_1$ and dopaminergic receptor antagonists as well as a serotonin synthesis inhibitor. On the other hand, tryimyristin exerted a depressant effect when tested in behavioral despair animal models and potentiated hypothermia induced by reserpine. The observed effects were blocked by pre-administration of a serotonin 5-HT$_{2A}$ receptor antagonist (Kasture and Gujar 2005). A previous study in our laboratory evaluated the neurobehavioral effects of nutmeg in the four-point tetrad assay as compared to common drugs of abuse, Δ^9-tetrahydrocannabinol (Δ^9-THC), morphine, and amphetamine. The results of the study showed that nutmeg extracts have various activities in the assay, depending on the nature of the extract, as well as the route of administration. The study demonstrated that the dichloromethane nutmeg extract, when injected at 100 and 300 mg/kg doses, i.p., exerted some cannabimimetic activity in the tetrad assay at (El-Alfy et al. 2009).

Unlike the plethora of preclinical studies evaluating the nutmeg extracts, very few studies examined the potential activities of nutmeg oil. Wahab et al. reported a dose-dependent activity of nutmeg oil in various animal seizure models (Wahab et al. 2009). The oil exerted significant anticonvulsant effect against seizures induced by maximal electroshock,

pentylenetetrazole, and strychnine, at doses that did not impair locomotor activity. Higher doses of the oil seem to possess a weak proconvulsant effect, potentiating the clonic seizures induced by both pentylenetetrazole and bicuculline. Administration of nutmeg essential oil to experimental animals via the inhalation route showed a dose-dependent depressant effect on locomotor activity, with potential sedative effect attributed to myristicin, safrole, and 4-terpineol oil constituents (Muchtaridi et al. 2010). Using the DPPH free radical scavenging assay, Piaru et al. reported a significant antioxidant activity of nutmeg oil (Piaru et al. 2012). The oil also showed strong cytotoxic action against colorectal cancer carcinoma cell line and breast carcinoma cell line.

Pharmacological evaluation of pure nutmeg compounds

In vitro & in vivo preclinical studies

Myristicin and trimyristin—Myristicin (**9**) is the major phenylpropanoid constituent of nutmeg essential oil. Various toxicological reports have attributed adverse effects associated with nutmeg ingestion to myristicin. These adverse effects include gastrointestinal; vomiting, and ileus, nervous system; drowsiness, paresthesia, numbness, reality detachment, and cardiovascular; hypotension, tachycardia, symptoms (Grover et al. 2002, Sangalli and Chiang 2000). Limited studies focused on preclinical pharmacological evaluation of myristicin. Leiter et al. examined the anxiolytic effect of myristicin in the experimental elevated plus maze (EPM) animal model (Leiter et al. 2011). In line with a previous report by Sonvane and co-workers, myristicin failed to show anxiolytic action in the Leiter et al. study (Sonavane et al. 2002). The results demonstrated that myristicin may actually have some anxiogenic effect and may antagonize the actions of benzodiazepine, the $GABA_A$ receptors allosteric potentiators. In addition the CNS activity, antihelmintic, insecticidal, apoptotic, and protection against DNA damage effects were reported to myristicin (Du et al. 2014, Lopez et al. 2015, Martins et al. 2014). However, most of these activities are scattered in the literature, with no systematic follow up studies that further corroborate the findings or carry them further to application.

The depressant effect of trimyristin, the main triglyceride constituent of nutmeg butter, was documented in mice (Kasture and Gujar 2005). Trimyristin significantly increased immobility time in both forced swim and tail suspension murine tests, indicating a depressant-like action, when administered i.p. at 10 and 30 mg/kg doses. Furthermore the activity was inhibited by various antidepressant medications including the selective serotonin reuptake inhibitor fluoxetine, the tricyclic antidepressants imipramine, and the atypical antidepressant mianserin. However, the mechanism of such depressant action remains unclear.

Lignans—The myriad uses of nutmeg, coupled to the complex mixture of compounds isolated, have triggered numerous pharmacological research efforts. The chemical groups, lignans and neolignans seem to be among the most studied. Nguyen et al., in search of novel AMP-protein kinase (5’-adenosine monophosphate-activated protein kinase, AMPK) activators, isolated 2,5-bis-aryl-3,4-dimethyltetarhydrofuran lignans **15**-**20** & **22** from nutmeg (Nguyen et al. 2010). AMPK enzyme system plays a crucial role in regulating lipid

and glucose homeostasis in a various tissue types. Studies have emphasized its role in obesity, diabetes, and cardiovascular diseases. Activation of AMPK has recently emerged as a therapeutic target for the aforementioned disease states (Lipovka and Konhilas 2015). Because of antihyperlipidemic and anti-atheroscelorotic activities reported for nutmeg extract (Sharma 2001), Nguyen et al. screened the seed extract of AMPK activator activity, followed by isolation of pure lignan compounds. Out of the seven isolated compounds, tetrahydrofuroguaiacin, nectandrin A (**18**), and nectandrin B (**19**) exerted strong activation of AMPK in differentiated C2C12 muscle cells, at 5 M concentration (Nguyen et al. 2010). The study also examined the effect of administration of a nectandrin B-rich active fraction in a high fat-induced animal model of obesity. The results showed a protective effect of the fraction against weight gain and blood glucose elevation caused by the high fat diet, suggesting potential application for nutmeg and its compounds in obesity, type 2 diabetes mellitus, and metabolic syndrome. A follow up study reported that nectandrin B activated AMPK in vascular smooth muscle cells (VSMC) and inhibited VSMC proliferation and neointima formation, events that are critical in the development of vascular occlusive diseases. An elaborate study of the mechanism of VSMC anti-proliferative effect revealed that AMPK activation resulted in P53 and P21induction, that in turn downregulated retinoblastoma (Rb) phosphorylation, E2 transcription factor 1 (E2F1) resulting in inhibition of pin1 gene expression. The cascade of events results in inhibition of intimal hyperplasia (Ki et al. 2013). These results support a therapeutic potential for nectandrin B in the treatment or prevention of various occlusive vascular diseases, however further studies, particularly clinical studies are still needed.

Reviewing the literature reveals that lignans have received wide attention for their potential role in prevention of osteoporosis. Lignans are classified as phytoestrogens that exert estrogenic activity through binding to the estrogen receptor. Various phytoestrogens, especially isoflavones, have demonstrated beneficial clinical effects against bone loss in both preclinical and clinical studies (Poluzzi et al. 2014). Machilin A (**31**), one of the lignan components of nutmeg, was reported to stimulate osteoblast differentiation through activation of the p38 mitogen activated protein (MAP) kinase pathway (Lee et al. 2009). In early stage osteoblast differentiation, machilin A significantly increase alkaline phosphatase (ALP) activity, a commonly used marker for stimulating differentiation. Similarly, machilin A activated late stage differentiation and significant bone mineralization. The observed bone anabolic activity occurred in a dose-dependent manner. Other activities for machilin A include inhibition of proliferation of blood lymphocytes, human leukemia HL-60 cells, and topoisomerase I and II inhibition, suggesting potential anticancer properties (Hirano et al. 1991, Hirano et al. 1994, Li et al. 2004). Follow-up studies and proper clinical evaluation of these properties remain uninvestigated.

Several pharmacological activities have been attributed to macelignan (**23**) the main bioactive component identified in nutmeg mace. The activities range from anti-microbial, anti-inflammatory, anti-cancer, to antidiabetic, hepato- and neuro-protective (Paul et al. 2013). The anti-inflammatory effects of macelignan have been extensively studied. Shin et al. reported that treatment with macelignan prevents the development of allergen-induced asthma in experimental animal models (Shin et al. 2013). The protective effect was coupled

to a reduction in CD4+ T cells production of interleukin-4 (IL-4), but with no apparent effect on IL-17 or interferon-cells. Animals administered macelignan showed lower expression of the type-2 T helper cell (T_h2) transcription factor, GATA3, an effect that needs might contribute to the protective anti-asthma activity, but requires further mechanistic studies. An earlier study demonstrated that macelignan inhibits the activation of mast cells in response to allergen exposure. Macelignan inhibited the release of histamine, calcium influx, degranulation, as well as various inflammatory mediators' release (Han et al. 2012). The protective effect of macelignan has been tested in a variety of models of neurological dysfunction. Cui et al. reported a protective effect of oral administration of macelignan against lipopolysaccharide (LPS)-induced hippocampus microglial cells in rats (Cui et al. 2008). It also protected against impaired spatial learning induced by chronic LPS administration, implying potential therapeutic benefit for Alzheimer's Disease (AD) patients. The mechanism of anti-inflammatory effect was investigated by Ma et al. (Ma et al. 2009). The study reported that macelignan suppressed LPS-induced activation of the Toll-like receptor 4 pathway, as evidenced by suppression of the nuclear factor NF-κB, reduction in cyclooxygenase type-2 (COX-2) expression, and inhibition of reactive oxygen species (ROS) generation. The results were in agreement with anti-inflammatory and protective effects of macelignan observed in animal models of diabetes and hepatotoxicity (Paul et al. 2013). A recent study corroborated the neuroprotective effect of macelignan. Using midbrain slice cultures, macelignan treatment protected dopaminergic neurons against the interferon (IFN)-γ and LPS-induced degeneration (Kiyofuji et al. 2015). Mechanistic studies revealed the protective neuroprotective effect observed was mediated by macelignan activation of the peroxisome proliferator activated receptor (PPAR-γ), which in turns activated arginase 1 enzyme expression. The result of the study implicates probable protective role of maceliganan against Parkinson's Disease (PD) and other neurodegenerative disorders. The use of macelignan as an antiphotoaging agent stems from its antioxidant, antionflammtory, as well as its documented ability to protect human skin fibroblasts against damaging effects of UVB irradiation. The observed protective effect is mediated by suppression of two cellular responses involved in premature skin aging: the upregulation of matrix metalloproteinases (MMPs) and reduced collagen synthesis (Lee et al. 2012). Another potential skin application for macelignan was reported by Choi et al. whereby the authors suggested the use of macelignan as a natural depigmenting agent based on its ability, at 10 M concentration, to inhibit melanosome transfer and dendrite formation in B16F10 melanoma cells (Choi et al. 2011). In addition to its earlier documented anti-diabetic effect, macelignan, isolated from *Schisandra grandiflora*, was recently reported to possess an inhibitory action against advanced glycation end products (AGEs), an effect that adds to its potential role in the management of diabetes and metabolic syndrome (Poornima et al. 2016).

Neolignans—For a long time studies have focused on lignans as the main active components of nutmeg. Recent research efforts explored the activities of neolignan-type compounds. Kang et al. reported an antiplatelet activity to *erythro*-(7*S*,8*R*)-7-acetoxy-3,4,3', 5'-tetramethoxy-8-*O*-4'-neolignan (EATN, **51**) (Kang et al. 2013). Results of the study showed that EATN exerted a concentration dependent inhibition of platelet aggregation induced by platelet activating-factor and thrombin, and arachidonic acid. EATN had IC_{50}

values of 3.2 ± 0.4 and 3.4 ± 0.4 M against platelet activating factor and thrombin-induced platelet aggregation, respectively. Further mechanistic investigation delineated the mechanism of anti-platelet activity. EATN regulates the level of cAMP, a crucial second messenger in the activation of platelet aggregation. EATN elevates intracellular cAMP levels inhibiting the Ca^{2+}-induced mobilization of platelets activated by thrombin. Though these results are promising and elude to potential therapeutic application of EATN in atherothrombotic diseases, *in vivo* and clinical studies are still in demand. Licarin E (**39**), another neolignan, proved to protect against UVB irradiation damage to human skin fibroblasts (Kwon et al. 2011). It reversed the two events induced by UVB: elevation of matrix metalloproteinase-1 (MMP-1) and reduction of procollagen expression. The molecular mechanism of these effects proved to be via stimulation of transforming growth factor (TGF)/Smad signaling pathway. Similar to macelignan, licarin E could offer a novel therapeutic agent for photoaging. Using rat basophilic leukemia cells, stimulated by dinitrophenyl-human serum albumin, the effect of the neolignan licarin A (**35**) on histamine release and mast cell activation was examined (Matsui et al. 2015). The results demonstrated that licarin A inhibited mast cell activation, as evidenced by inhibiting tumor necrosis factor (TNF), COX-2, and prostaglandin (PGD2) production. Further studies are needed to corroborate the role of licarin A in the treatment of immediate hypersensitivity cases. *In vitro* studies have elucidated that licarin A, isolated from *Machilus thunbergii*, may possess neuroprotective value against glutamate-induced toxicity of rat cortical cells (Ma et al. 2005). The protective effect was also evident against kainic acid-induced neurotoxicity, though more selective protection was observed for glutamate toxicity. The neuroprotective effect was attributed to the potent antioxidant properties of licarin A, evidenced by reduction of NO, peroxide, free radical production as well as enhancing the activity of antioxidant enzyme systems. In addition, licarin A effectively suppressed Ca^{2+} influx that is typically induced by glutamate. Synthetic (−)-licarin A was reported to possess concentration-dependent anti-parasitic activity (Neris et al. 2013). *In vitro* studies reported potent inhibition of growth *Leishmania promastigotes*, suggesting promising application as a leishmanicidal agent. Using a tuberculosis murine animal model, Leon-Diaz et al. demonstrated that licarin A possesses a significant suppressant action of the pulmonary burden and pneumonia in animals infected with both drug sensitive as well as drug-resistant tuberculosis strains (Leon-Diaz et al. 2013). Animals administered licarin A (5mg/kg for 30 days) showed significant reduction in lung bacilli and pneumonia incidence. The anti-parasitic effect of licarin A was further corroborated against both *Schistosoma mansoni* and *Trypanosoma cruzi* (Pereira et al. 2011). It is evident that the potential anti-parasitic activity of licarin A is worth further investigation in the hope of future development of effective medications.

Diphenylalkanes—Maity and co-workers reported a significant healing effect of malabaricone B (**59**) against indomethacin-induced stomach ulcer (Maity et al. 2009). Administration of malabaricone B attenuated the increased nitric oxide synthesis induced by indomethacin, while enhancing the arginase pathway, thus favoring an anti / pro inflammatory cytokine ratio. Malabaricone C (**60**) has potential pharmacological benefits in vascular disease (Lee et al. 2012), promotion of healing, anti-inflammation and angiogenesis caused by stomach ulcers (Banerjee et al. 2008, Banerjee et al. 2008, Maity et al. 2009),

antioxidant activity (Patro et al. 2005) and cytotoxic activity against certain cancers (Patro et al. 2010, Tyagi et al. 2014). Malabaricone C also has anti-anaerobic, antifungal, and antibacterial properties (Chong et al. 2011, Orabi et al. 1991, Shinohara et al. 1999) as well as anti-parasitic, leishmanicidal and nematocidal activity (Hosoi et al. 1999, Sen et al. 2007).

Clinical trials

While phytochemical studies have supported some of the folkloric uses of nutmeg, very few clinical studies systematically investigated the clinical efficacy of its use. A thorough literature search revealed merely two small clinical studies. A randomized, placebo-controlled, double blind trial examined the clinical effects of using topical nutmeg extract in patients with painful diabetic neuropathy (Motilal and Maharaj 2013). The study included 74 diabetic neuropathy patients (males and females, ages 30–85 years) who were randomized to receive the different topical treatment: nutmeg extract, mace oil, nutmeg oil, coconut oil, methyl salicylate, menthol, or placebo. The study used a validated Brief Pain Inventory that has been modified for painful diabetic neuropathy in addition to the Neuropathic Pain Symptom Inventory. Following four weeks of treatment, patients showed within group significant improvement of pain, mood scores, and daily functions. However no statistically significant effect was reported between the nutmeg treated and placebo groups. Being the only study that assessed the clinical analgesic effect of nutmeg, it is hard to draw conclusions because of the study limitations. The small sample size, short duration of the study, lack of inert placebo, poor patient compliance, and use of non-standardized nutmeg preparations are all limitations that hinder proper evaluation of the therapeutic role of nutmeg in pain disorders. The other study retrieved describes an open, uncontrolled trial that examined the effect of administration of a nutmeg-containing herbal product in a total of 251 patients (Naidu et al. 1997). The product used, Revivin, contained a mixture of various plant extracts including nutmeg in addition to carbohydrate molecules, and is commonly used to enhance performance, improve appetite, and reduce weakness and fatigue. Patients (average age 44 years, males and females) received the capsule daily for 4 weeks. Outcomes were assessed by self-filled patients' questionnaire. Patients reported improvement in mood, insomnia, and overall weakness, and no adverse effects were reported by the patient. The study suffers from several limitations: subjectivity of outcome evaluation, short duration, and lack of placebo control. In addition, since the study used a product where nutmeg constituted only one component of the mixture, the clinical nutmeg effect cannot be isolated. As evident, there is a lack of well-designed controlled clinical trials that evaluate the potential therapeutic place of nutmeg and its components.

Conclusion

Nutmeg kernel and mace have a long history of use as a spice and traditional remedy that goes back to the 12th century. Traditional uses of nutmeg in alleviating gastrointestinal disorders, managing rheumatic pain, healing skin wounds and infections as well as its use as a calming agent resulted in massive contemporary efforts to evaluate its different extracts, fractions. One of the most commonly evaluated activities of nutmeg essential oil and extracts is their effect on the CNS. In addition to the essential oil which is rich in terpenes and phenylpropanoids, nutmeg has significant levels of non-volatile secondary metabolites of the

lignan/neolignan-type as well as diarylalkanes that have been isolated and identified. GC and GC/MS methods are routinely used for analysis of nutmeg essential oil while HPLC, HPTLC and LC-MS are more commonly used for fingerprinting of total extracts and bioanalysis of individual constituents. Total synthetic methods have also been developed for the preparation of some of the most biologically active constituents of nutmeg belonging to the above mentioned classes. Preclinical studies to evaluate such compounds as myristicin, macelignan, nectandrin A & B, licarin A-E, and malabaricone B & C are abundant in recent literature with focus on anxiolytic, antioxidant/chemopreventive, anti-inflammatory, anti-infective effects of individual compounds. On the other hand, clinical trials are not as abundant in the current literature. They are limited in size, design and focus (topical application of nutmeg or use of a mixed herbal product containing nutmeg among other extracts) with no individual pure compounds included. As a source of diverse secondary metabolites with significant potential as prototype agents for drug discovery, nutmeg needs more attention on a number of frontiers including constant supply of bioactive compounds through analytical method development, reproducible isolation procedures and efficient synthetic methods for promising secondary metabolites. Beyond the supply issue, further evaluation of bioactive constituents is warranted especially in *in vitro* mechanistic studies and functional assays leading to *in vivo* pharmacokinetic/dynamic evaluation resulting in further development towards new drug entities of benefit to mankind.

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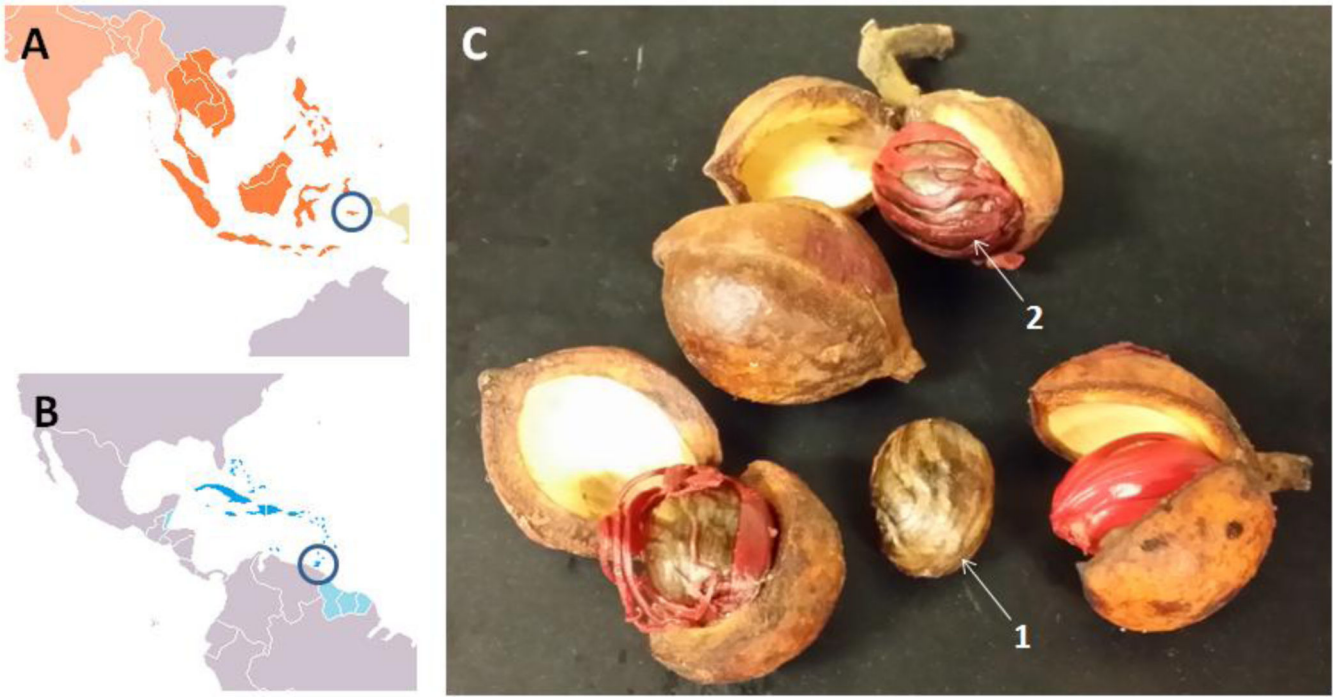


Figure 1.

Geographical locations and morphological structure of nutmeg*. (A) East Indies (Maluku islands circled); (B) West Indies (Grenada islands circled); (C) nutmeg fruit, seed kernel (1) and arillus (2). **(West Indies tree droppings)*

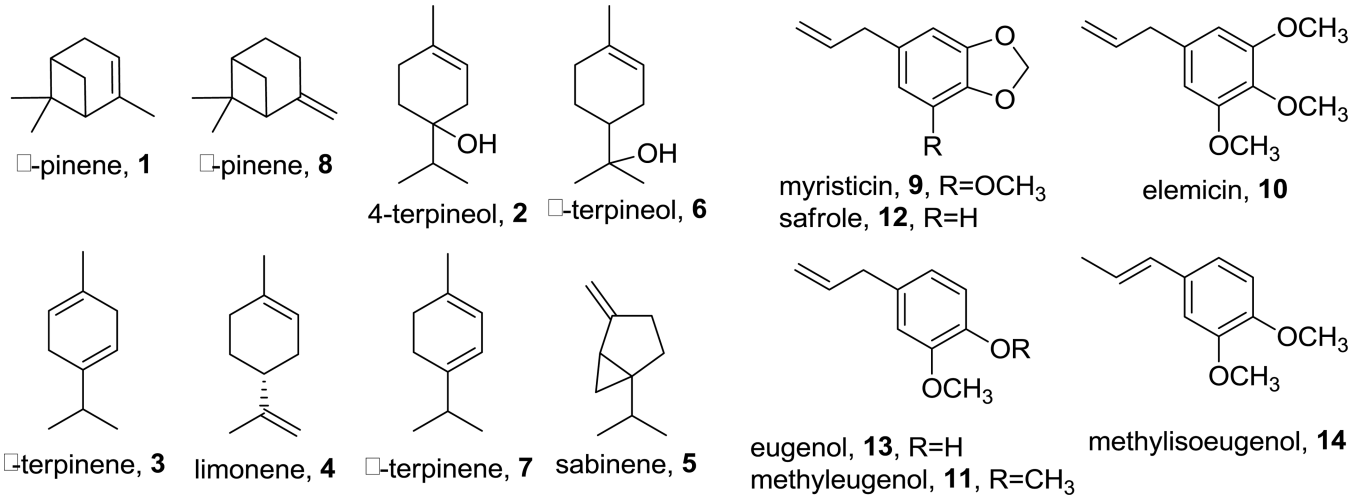


Figure 2.
Significant monoterpenes and phenylpropanoids of nutmeg essential oil

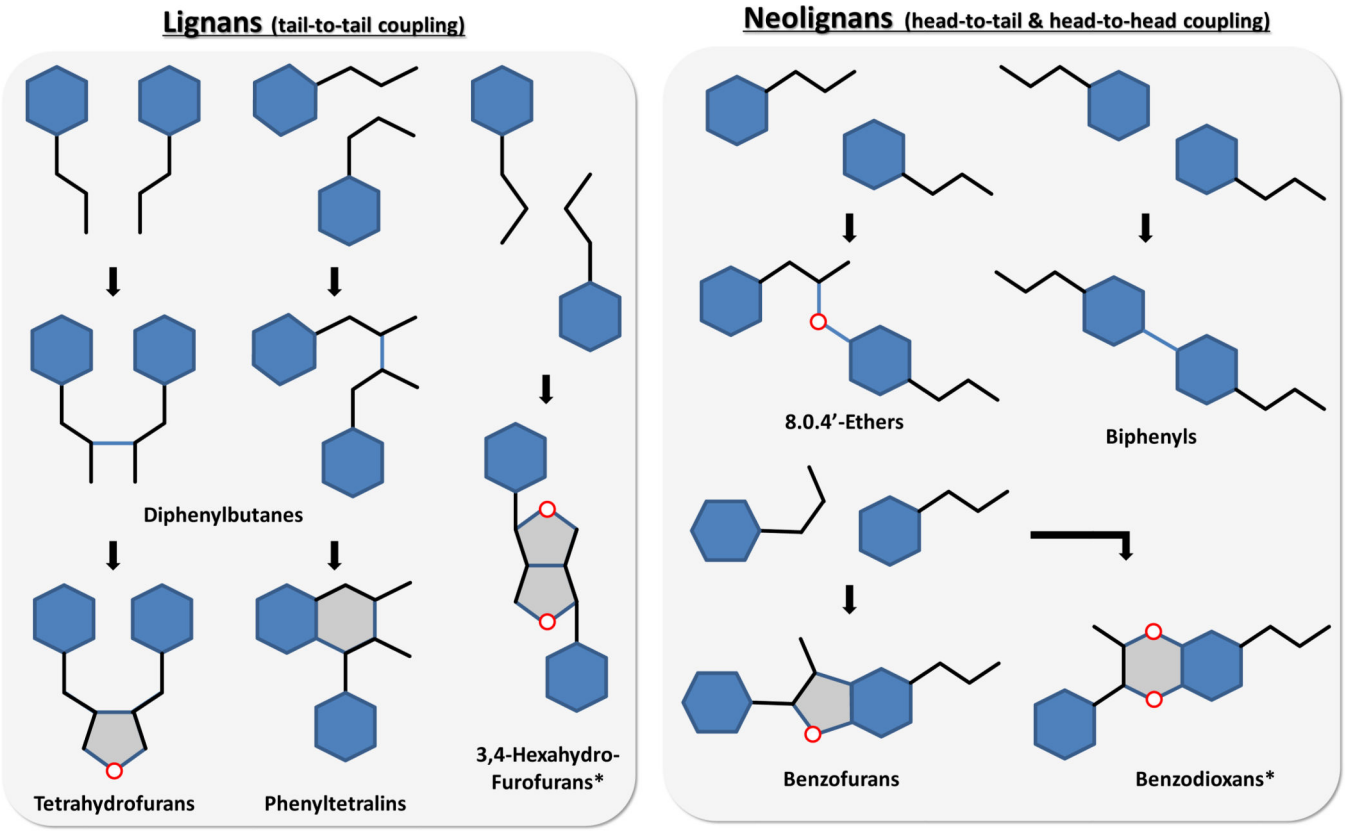
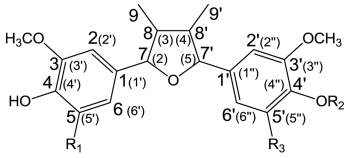
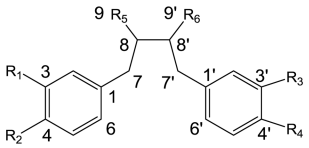


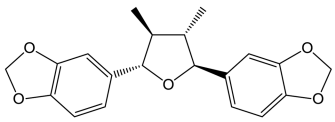
Figure 3.
Phenylpropanoid coupling patterns leading to formation of lignans and neolignans. **have not been reported in nutmeg*



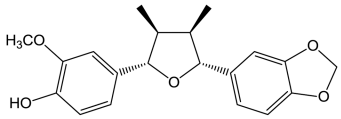


tetrahydrofuroguaiacin B, **15**; 2*S*,3*R*,4*S*,5*R*; R_1=R_2=R_3=H
saucernetindiol, **16**; 2*R*,3*S*,4*S*,5*R*; R_1=R_2=R_3=H
verrucosin, **17**; 2*R*,3*S*,4*S*,5*S*; R_1=R_2=R_3=H
nectandrin A, **18**; 2*S*,3*S*,4*R*,5*R*; R_1=R_3=H, R_2=CH_3
nectandrin B, **19**; 2*S*,3*S*,4*R*,5*R*; R_1=R_2=R_3=H
fragransin C1, **20**; 2*S*,3*S*,4*R*,5*R*; R_1=OCH_3, R_2=H
epoxylignan 1, **21**; 2*S*,3*S*,4*R*,5*R*; R_1=R_2=H, R_3=OCH_3

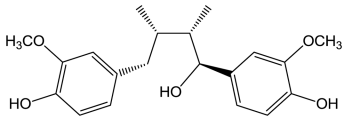
macelignan, **23**, 8*S*,8'*R*; R_1=OCH_3, R_2=OH, $R_3{}^+R_4$=OCH_2O
diphenylbutane 1, **24**, R_1=R_2=OH, $R_3{}^+R_4$=OCH_2O, R_5=R_6=CH_3
9'-hydroxymachilin A, **25**, 8*R*,8'*S*; $R_1{}^+R_2$=OCH_2O, $R_3{}^+R_4$=OCH_2O, R_5=CH_3, R_6=CH_2OH
monomethyldihydroguaiaretic acid, **26**, R_1=R_3=R_4=OCH_3, R_2=OH, R_5=R_6=CH_3
meso-dihydroguaiaretic acid, **30**, 8*R*,8'*S*; R_1=R_3=OCH_3, R_2=R_4=OH
machilin A, **31**, 8*R*,8'*S*; R_1+R_2=OCH_2O, R_3+R_4=OCH_2O, R_5=R_6=CH_3



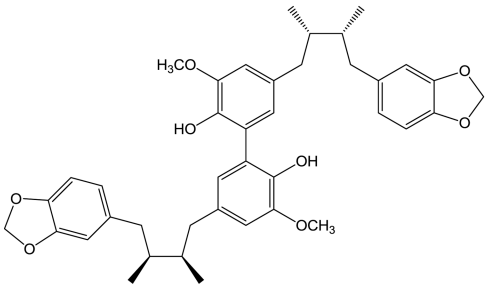
galbacin, **22**



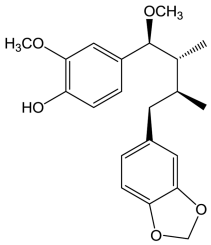
machilin F, **32**



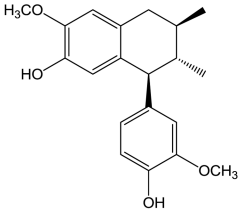
myristargenol, **33**



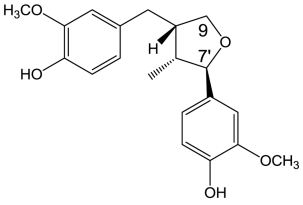
argenteane (from *M. fatua*), **34**



diphenylbutane 3, **27**

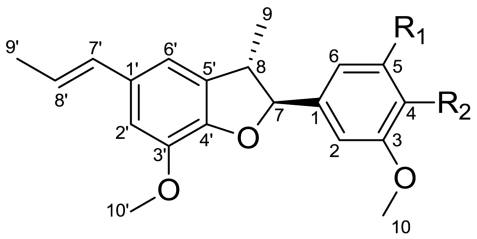


(+)-guaiacin, **28**

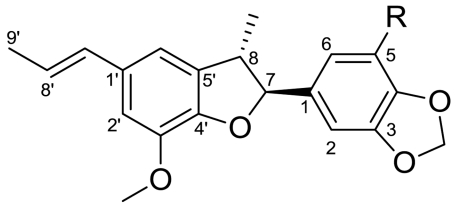


7',9-epoxylignan 1, **29**

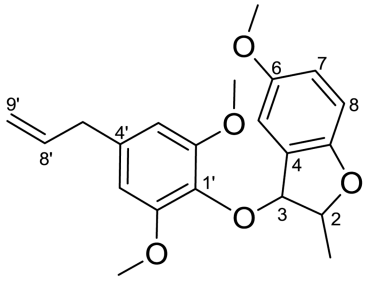
Figure 4.
Lignan secondary metabolites identified in *M. fragrans* and other species of *Myristica*



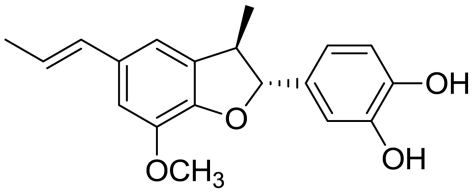
licarin A, **35**, R_1=H, R_2=OH
licarin C, **36**, R_1=R_2=OCH_3



licarin B, **37**, R=H
5-methoxylicarin B, **38**, R=OCH_3
licarin E, **39**, (7*R*,8*R*) enantiomer of licarin B

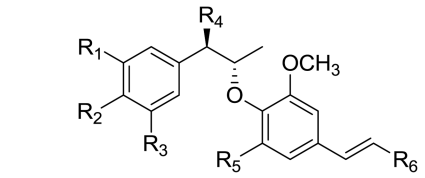


3-(4'-allyl-2',6'-dimethoxy-phenoxy)-2-methyl-6-methoxy-2,3-dihydro-benzofuran, **41**

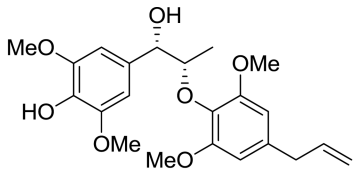


(7*R*,8*R*)-7,8-dihydro-7-(3,4-dihydroxyphenyl)-3'-methoxy-8-methyl-1'-(*E*-propenyl)benzofuran, **40**

A.



	R_1	R_2	R_3	R_4	R_5	R_6
42	OCH_2O		H	OH	OMe	CH_3
43	OMe	OMe	H	OAc	OMe	CHO
44	OMe	OH	OMe	OH	H	CH_3
46	OMe	OH	OMe	OH	OMe	CHO
47	OMe	OMe	OMe	OH	H	CH_3



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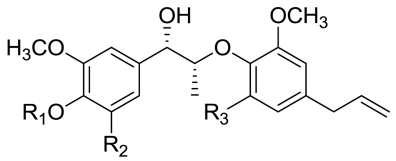
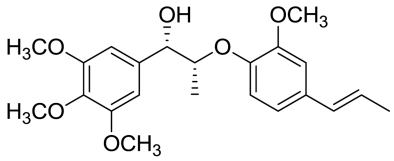
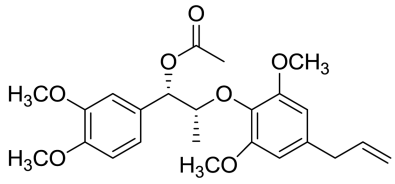


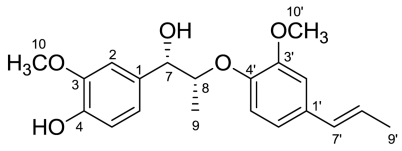
myrislignan, **48**, $R_1=R_2=R_3=H$
raphidecursinol B, **49**, $R_1=CH_3$, $R_2=R_3=OCH_3$



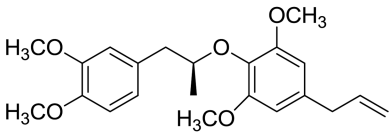
surinamensin, **50**



(+)-*erythro*-(7*S*,8*R*)-□[8']-7-acetoxy-3,4,3',5'-tetramethoxyphenyl)-8-O-4'-neolignan, **51**



erythro-(7*S*,8*R*)-7-(4-hydroxy-3-methoxyphenyl)-8-[2'-methoxy-4'(*E*)-propenyl)phenoxy]propan-7-ol, **52**



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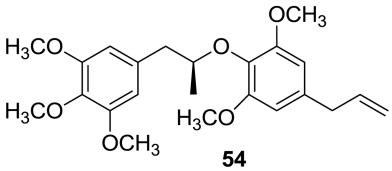

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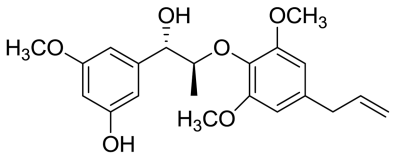

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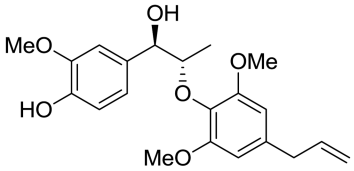

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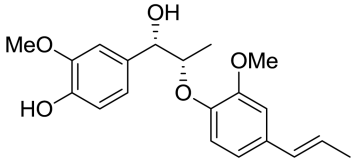

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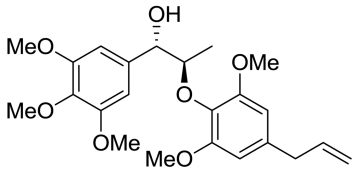


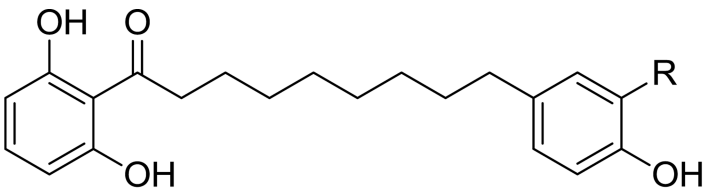
58

B.

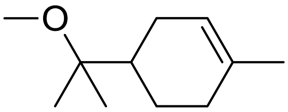
Figure 5.

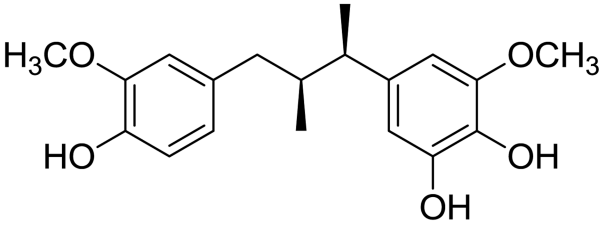
A. Benzofuranoid neolignans of nutmeg

B. Non-benzofuranoid (8.*O*.4') neolignans (8,4'-oxyneolignans) of nutmeg

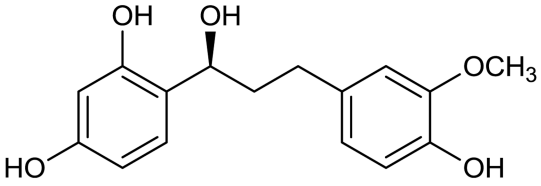


malabaricone B, **59**, R=H,
malabaricone C, **60**, R=OH
61, R=

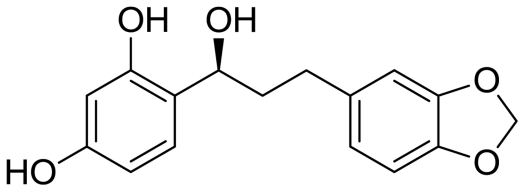




diphenylpropane 1, **62**

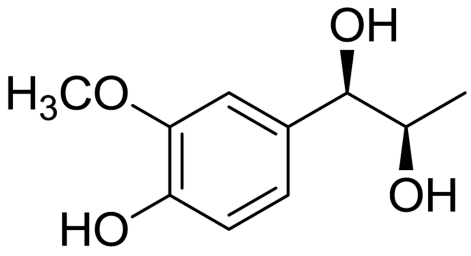


diphenylpropane 2, **63**

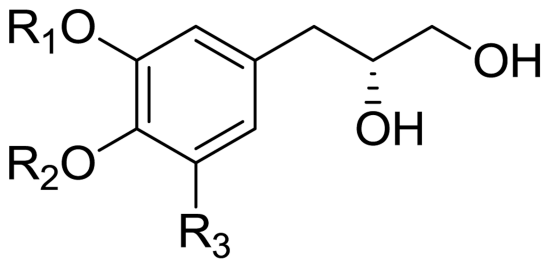


diphenylpropane 3, **64**

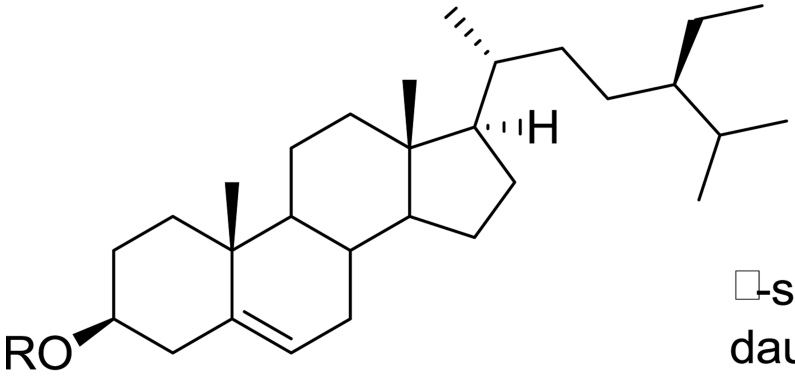
Figure 6.
Diphenylalkanes isolated from *Myristica fragrans*



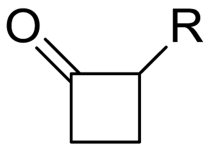
65



66, $R_1=R_2=CH_3$, $R_3=OCH_3$
67, $R_1=R_2=CH_2$, $R_3=OCH_3$
68, $R_1=R_2=CH_2$, $R_3=H$



□-sitosterol, **69**, R=H
daucosterol, **70**, R=glucosyl



2-decylcyclobutanone, **71**, R=$(CH_2)_9CH_3$
2-dodecylcyclobutanone, **72**, R=$(CH_2)_{11}CH_3$

Figure 7.
Phenylpropanediols, steroids and alkylcyclobutanone secondary metabolites detected in nutmeg kernel

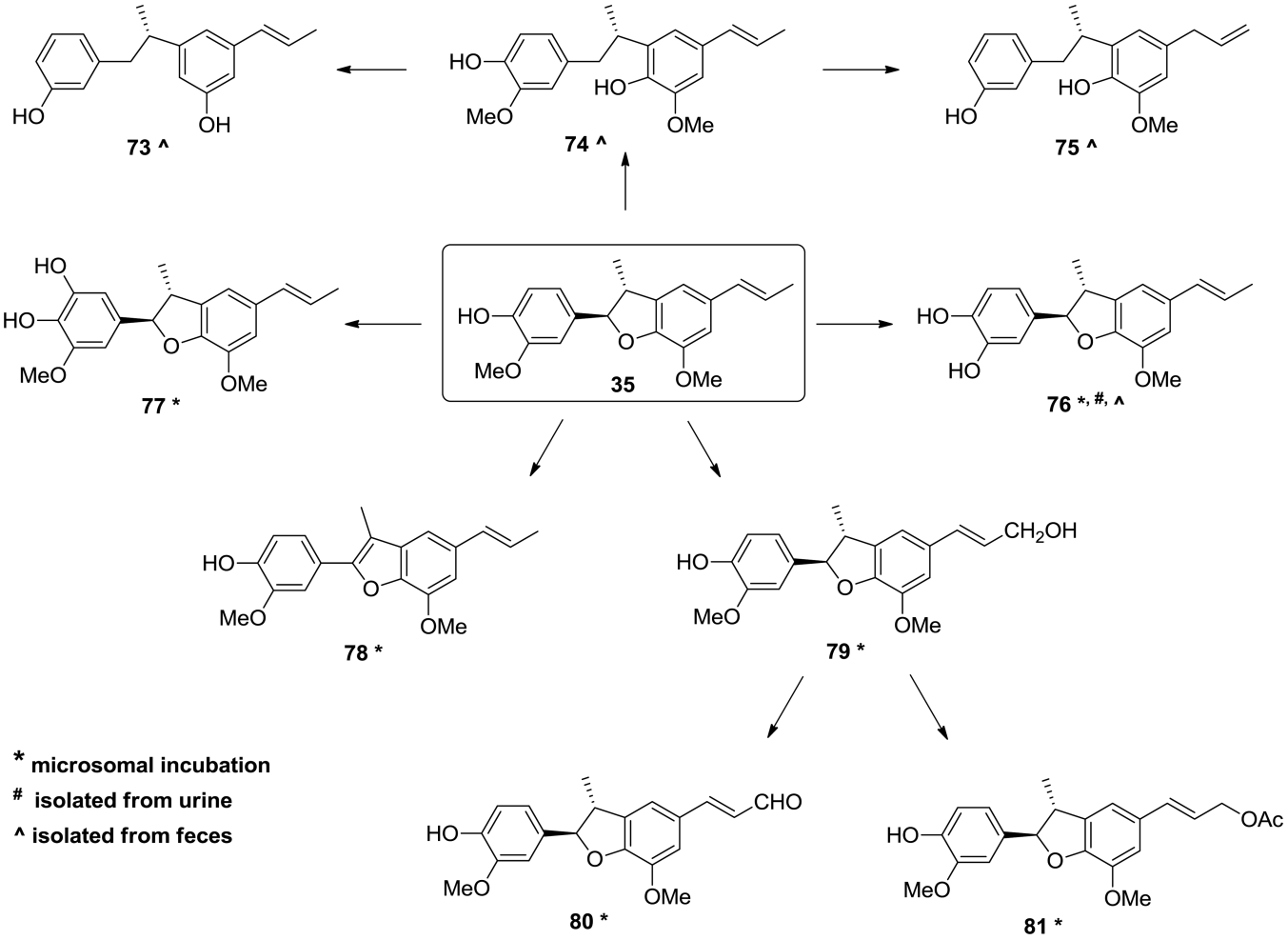
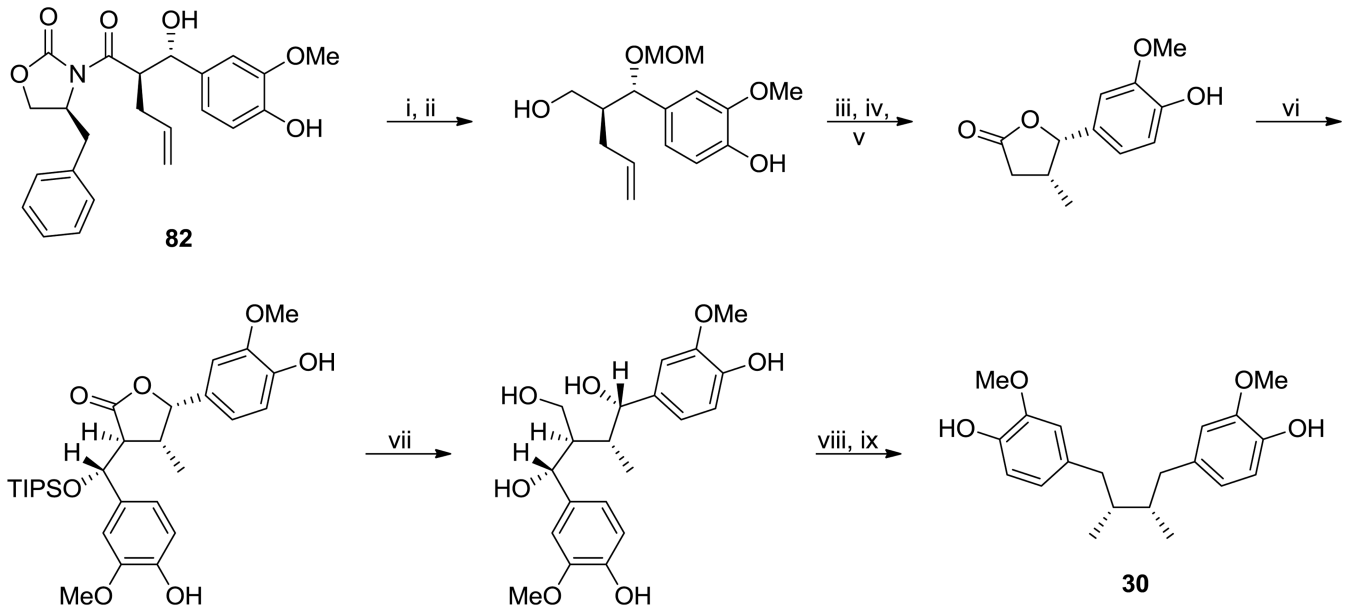


Figure 8.
Rat metabolism of licarin A (**35**)(Li and Yang 2011)



i) MOMCl, (*iso*-Pr)$_2$NEt, CH_2Cl_2, 4h. ii) $LiAlH_4$, MeOH, THF, 60 ^oC, 6h. iii) *p*-TsCl, Pyr., CH_2Cl_2, 20h; iv) $LiAlH_4$, THF, 20h. v) 1. OsO_4, NMO, aq. acetone, *tert*-BuOH, 18h; 2. $NaIO_4$, MeOH, 1h; 3. 2-methyl-2-butene, $NaH_2PO_4.2H_2O$, $NaClO_2$, aq. *tert*-BuOH; 4. 6M aq. HCl, THF, 50 ^oC, 20min. vi) 1. KHMDS, 4-benzyloxy-3-methoxybenzaldehyde, THF, -70 ^oC, 1h (*erythro*/*threo* = 1/3); 2. TIPSOTf, 2,6-lutidine, CH_2Cl_2, 1h. vii) 1. DIBAL-H, toluene, -70 ^oC, 3h; 2. $NaBH_4$, EtOH, 1.5h; 3. 6M aq. HCl. viii) 1. TsCl, Pyr., CH_2Cl_2, 18h; 2. $NaBH_4$, HMPA, 13h. ix) H_2, 10% $Pd(OH)_2$/C, THF, 70h.

Figure 9.
Total synthesis of the lignan dihydroguaiaretic acid (**30**)(Kawaguchi et al. 2009)

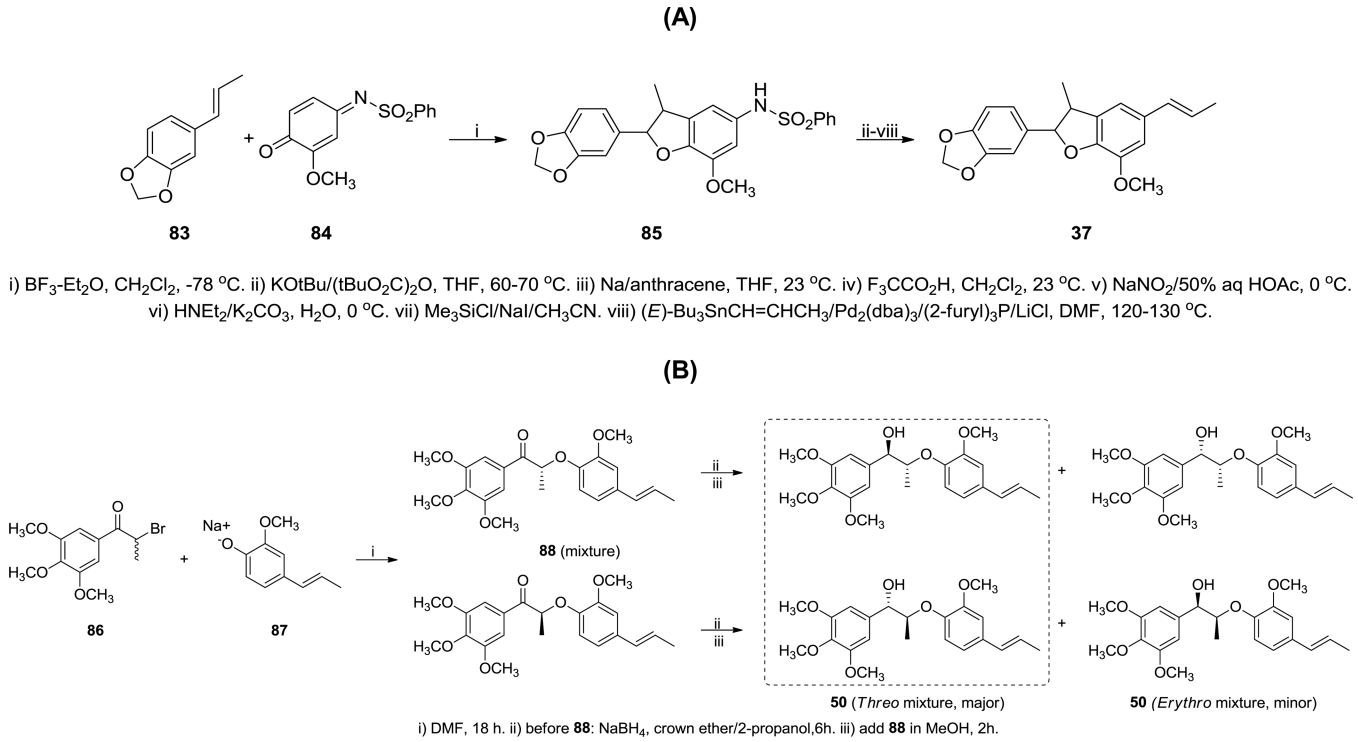
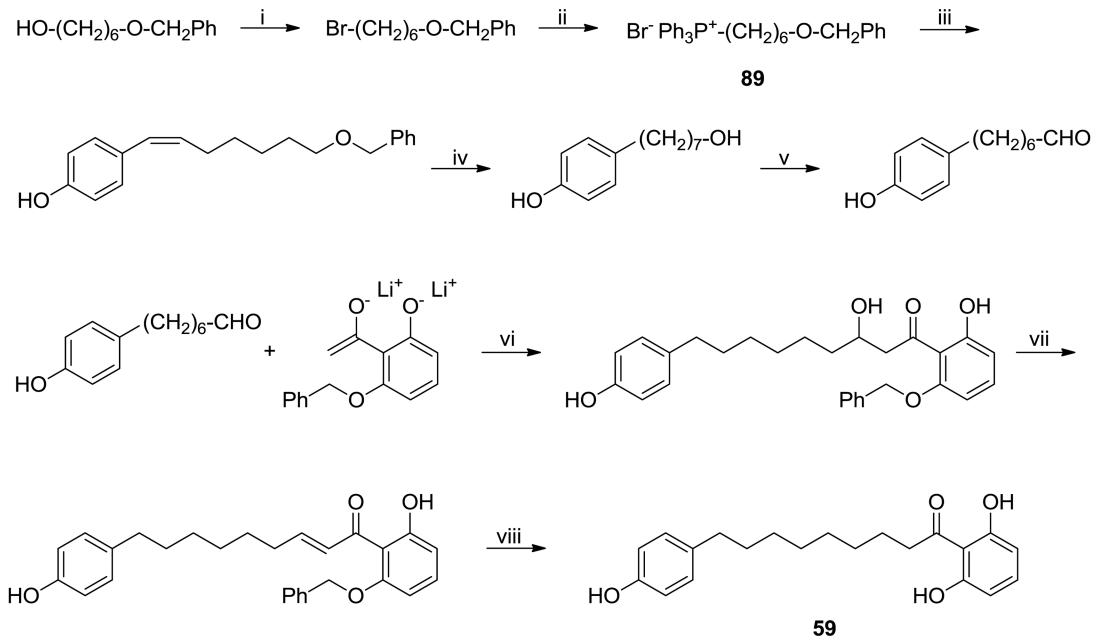


Figure 10.

Total synthesis of selected neolignans. A. Licarin B (**37**) (Engler and Chai 1996); B. Surinamensin (**50**) (Zacchino and Badano 1985)



i) Ph_3P/NBS, THF, 2h. ii) Ph_3P, 2h, 140 ^oC. iii) 1. tert-BuOK/THF-DMSO; 2. *p*-hyroxyphenyl-CHO, 50 min, Ar. iv) H_2/Pd-C (10%), EtOH, 4 kg/cm^2, 24 h. v) Swern oxidation 1. DMSO/oxalyl chloride, CH_2Cl_2, 15 min, -78 ^oC; 2. Et_3N, 5 min then rt, 15 min. vi) THF/HMPA, -78 ^oC, 1h. vii) *p*-TsOH, benzene, reflux, 1h. viii) H_2/Pd-C (10%), acetone, 3.5 kg/cm^2, 6h.

Figure 11.

Total synthesis of malabaricone B (**59**) (Hosoi et al. 1999, Tsuda et al. 1991)

Table 1

Relative chemical composition of nutmeg kernel and methods of preparation of major constituents (Abourashed and Khan 2010, Daniel 1994, Olaleye et al. 2006)

Chemical class	Concentration (w/w, dry)	Main Constituents (abundance within class)	Preparation Method
I) Primary metabolites:			
Fixed oil (nutmeg butter)	up to 40%	Myristic acid (~8%) & trimyristin triglyceride (~73%)	Expression
Carbohydrates	up to 30%	Starch	Insoluble residue
Protein	up to 6%		Insoluble residue
II) Secondary metabolites:			
Essential oil	2–16%	Terpenes (~88%), Phenylpropanoids (~12%)	Steam distillation
Small phenolic compounds	varies	Phenolic acids, lignans, diarylalkanes, flavonoids	Organic solvent extraction
Resins & pigments	varies	Polyphenolics, polycatechins, tannins, anthocyanins	Variable

Table 2

HPLC and HPTLC methods for analysis of nutmeg constituents in crude extracts and herbal preparations

Application	Technique/Method	Target Constituents	Ref.
Quality control of kernel and mace oil obtained by supercritical fluid extraction	RP HPLC-UV: Lichrosphere 100, C18, 125 × 4 mm, 5 ; gradient MeOH/ACN/0.01% aq. PA; 1 mL/min; 23 min; 265 nm	9 Phenylpropanoids: ele, eug, ieug, ielem, mxeug, myeug, myr, saf	(Ehlers et al. 1998)
Determination of bioactive constituents in crude extracts and market products of fruit	RP UPLC-UV: Acquity BEH, C18, 1.7, 50 × 2.1 mm; gradient MeOH/0.1% aq. FA (10–100%); 0.4 mL/min; 9.4 min; MRM MS pos-neg mode switching	16 compounds: apg, cafa, cat, fera, eug, ieug, irhm, kmf, myeug, ma, myr, ola, ursa, pca, qrc, trm	(Pandey et al. 2015)
Fingerprinting and guided isolation of kernel constituents	RP HPLC-UV: HyPurity, C18, 3, 150 × 4.6 mm; gradient ACN/0.1% FA; 1 mL/min; 30 min; 270 nm	13 compounds: acneol, dhb, elem, ielem, licA, licB, licC, malB, malC, mlicA, mlicB, myr, sur	(Chiu et al. 2016)
Pharmacognostical standardization of kernel extract and quality control of Indian products	NP HPTLC-UV: silica gel 60 F_{254} coated plates; toluene:ethyl acetate (9:1); 254 nm; 366 nm; *p*-anisaldehyde/H_2SO_4	17 spots: Only myr identified	(Tripathi and Dwivedi 2015)

UHPLC: Ultra-performance liquid chromatography; HPTLC: high-performance thin-layer chromatography; NP: normal phase; RP: reversed phase; UV: ultraviolet detector; MS: mass spectrometry detector; acetoxyneolignan (**51**): acneol; apigenin: apg; caffeic acid: cafa; catechin: cat; dihydrobenzofuran (**41**): dhb; elemicin (**10**): elem; eugenol (**13**): eug; ferulic acid: fera; isoeugenol: ieug; isoelemicin: ielem; isorhamnetin: irhm; kaempferol: kmf; licarin A (**35**): licA; licarin B (**36**): lic B; licarin C (**37**): licC; malabaricone B (**59**): malB; malabaricone C (**60**): malC; 5-methoxylicarin A: mlicA; 5-methoxylicarin B (**38**): mlicB; methoxyeugenol: mxeug; methyleugenol (**11**): myeug; methylisoeugenol (**14**): myieug; myristic acid: ma; myristicin (**9**): myr; oleanolic acid: ola; ursolic acid: ursa; protocatechuic acid: pca; quercetin: qrc; safrole (**12**): saf; surinamensin (**50**): sur; trimyristin: trm; ACN: acetonitrile; MeOH: methanol; FA: formic acid; PA: phosphoric acid