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A Review on Chemical Composition, Bioactivity, and Toxicity of *Myristica fragrans* Houtt. Essential Oil

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ABSTRACT

Myristica fragrans Houtt., commonly known as nutmeg, is an Indonesian indigenous dioecious evergreen tree that contains 5-15% volatile oil. The oil is usually produced from the seed or mace. Nutmeg oil has been extensively utilized in aromatherapy, natural medicine, and the perfume industry. This article provides an overview of the chemical compounds, biological potency, and toxic effects of nutmeg essential oil compiled from recent literature (2000–2020). Nutmeg oil mainly comprises monoterpenes and phenylpropanoids. Several reports on gas chromatography-mass spectrophotometry analysis of nutmeg oil showed that there were 27–38 chemical constituents detected at various concentrations. Many secondary metabolites of nutmeg oil are reported to show biological activities that possibly substantiate its utilization in natural medicine. Numerous studies reported the biological activities of this volatile oil such as antioxidant, analgesic, antiinflammation, anticonvulsant, antibacterial, antiparasitic, insecticidal, and anticancer activity. But large intake of nutmeg oil could cause intoxication which is shown through symptoms in the cardiovascular, central nervous system, anticholinergic, and local effects in the stomach. These symptoms are mainly attributed to the effect of myristicin, safrole, and elemicin overdose. This updated review paper intends to attract more attention to nutmeg oil and its potential to be developed into a medicinal product for the prophylaxis and therapy of diseases.

Keywords: Nutmeg, volatile oil, chemical composition, bioactivity, toxicity.

INTRODUCTION

Myristica fragrans Houtt. or nutmeg is an indigenous plant of Indonesia (Ibrahim *et al.*, 2020; Rahardiyan *et al.*, 2020). It has been cultivated in many tropical countries such as Malaysia, India, Indonesia, Grenada, Mauritius, Singapore, Sri Lanka, and many African countries (Al-Rawi *et al.*, 2011; Kuete, 2017). Nutmeg is a unique kitchen spice with a distinct fragrance and warm, slightly sweet taste. It had been used traditionally as a natural remedy in Tibet, China, India, Indonesia, Arabs of Israel, and Jewish (Periasamy *et al.*, 2016) to treat digestive problems, such as indigestion, flatulence, diarrhea, and colic, insomnia, urinary incontinence, dyspepsia, arthritis, cold, headache, pyocutaneous disease, and asynodia (Zheljazkov *et al.*, 2015; Abourashed and El-Alfy, 2016; Periasamy *et al.*, 2016; Zhao *et al.*, 2017).

Nutmeg yields 5-15% of volatile oil (Stein *et al.*, 2001; Periasamy *et al.*, 2016). The major constituents of nutmeg essential oil (NEO) are terpenes and alkenyl benzene derivates (Abourashed and El-Alfy, 2016). In folkloric medicine, NEO has been practically applied as a topical preparation to treat rheumatism, since it possesses analgesic and anti-inflammatory effects (Asgarpanah, 2012). In recent years, NEO has been found to have various biological properties e.g., antioxidant (Piaru *et al.*, 2012; Adiani *et al.*, 2015; Sipahelut *et al.*, 2020), analgesic, antiinflammation (Zhang *et al.*, 2016), anticonvulsant (Wahab *et al.*, 2009), antiinfection (Gupta and Rajpurohit, 2011; Pillai *et al.*, 2012), insecticidal (Du *et al.*, 2014; Norris *et al.*, 2015; Soni *et al.*, 2016), and anticancer (Piaru *et al.*, 2012b).

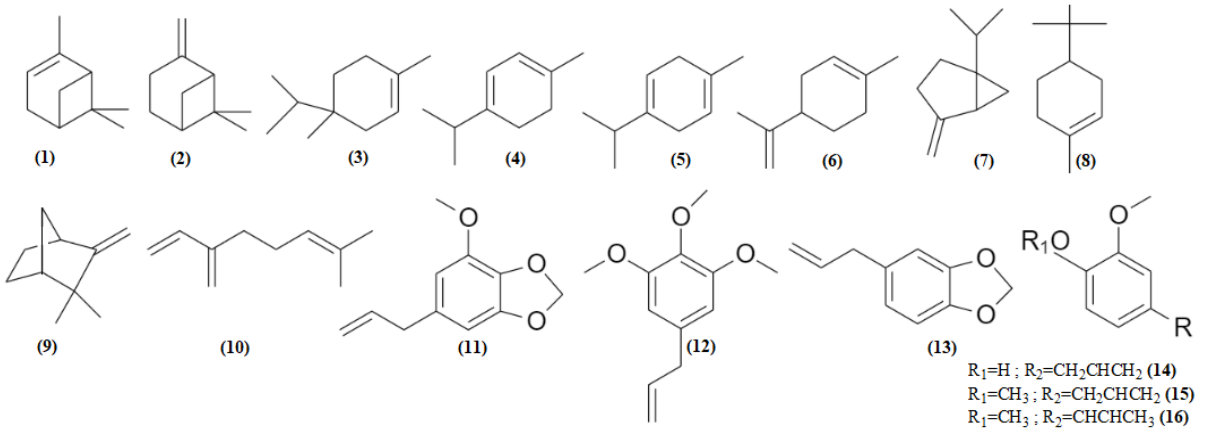

Figure 1. The goodness of fit of the one-compartment open model of levofloxacin after intravenous bolus injection, i.e., individual data fitting analyses (panel A); observation versus population prediction analysis (panel B) and observation versus individual prediction analysis (panel C).

Several review articles had been reported on nutmeg botanical aspect, secondary metabolites, the analytical methods used to analyze the extracts and pure compounds, phytochemical aspect, the total synthesis process of some major constituents, and the pharmacological activities of nutmeg extract (Asgarpanah, 2012; Abourashed and El-Alfy, 2016; Kuete, 2017). While presently, review on NEO is still limited about its toxicological aspect (Ehrenpreis *et al.*, 2014). Therefore, this article will highlight the scientific reports of NEO chemical composition, bioactivities, and toxicity, with special attention to bioactive compounds and the mechanism underlying their effect.

MATERIALS AND METHODS

This review utilizes narrative review in favor of its perceived strength in identifying accomplishments and gaps of previous studies (Rother, 2007; Grant and Booth, 2009). A web-based purposive literature search was conducted by using '*Myristica fragrance* in combination with: 'nutmeg', 'essential oil', 'chemical composition', 'bioactivity', and 'toxicity' as the keywords. The searching process was limited to the recently published literature from 2000 to 2020.

RESULT AND DISCUSSION

This review summarized pharmacological studies on NEO over the last 20 years (2000-2020). This review particularly focused on NEO chemical composition, biological activities, and toxicity to compile NEO wholesome profile for the sake of NEO development into medicinal products with diverse pharmacological properties.

Chemical composition

NEO is rich in monoterpenes (about 90%) and phenylpropanoids (Abourashed and El-Alfy, 2016). Several reports on GC/MS analysis of NEO showed that there were 27–38 compounds detected at various concentrations (Abourashed and El-Alfy, 2016). Kapoor *et al.* (2013) identified 38 compounds in NEO. While two individual studies conducted by Wahab *et al.* (2009) and Piaru *et al.* (2012) successfully identified 37 chemical compounds in the NEO. While in other studies, Muchtaridi, et al. (2010) recorded 32 constituents, Piras *et al.* (2012) reported 30 compounds, and Du *et al.* (2014) identified 27 compounds. Thus, the average number of chemical constituents in NEO detected based on these studies is 34.

The monoterpenes that are commonly identified in NEO are α-pinene **(1)**, β-pinene **(2)**, 4-terpineol **(3)**, α-terpinene **(4)**, γ-terpinene **(5)**, limonene **(6)**, sabinene **(7)**, α-terpineol **(8)**, camphene **(9)**, and myrcene **(10)**. Whereas the phenylpropanoids compounds that can be found are myristicin **(11)**, elemicin **(12)**, safrole **(13)**, eugenol **(14)**, methyl eugenol **(15)**, and methyl isoeugenol **(16)** (Figue 1).

Biological activities

NEO has various pharmacological properties and active constituents, such as α-pinene, elemicin, 4-terpineol, myristicin, eugenol, safrole, and linalool (Table I).

Table Ia. Myristica fragrans Houtt. essential oil bioactivities

Biological activities	Study model	Assay	Concentration or dose	Active constituent of NEO	Ref.
Antioxidant	*In vitro*	DPPH	2.94±0.09μM g^{-1} TEAC	Elemicin, 4-terpineol, myristicin, trans-sabinene hydrate, safrole, eugenol, β-caryophyllene, isoeugenol, sabinene, α-pinene, limonene, and α-terpinene	(Gupta and Rajpurohit, 2011; Piaru *et al.*, 2012b; Adiani *et al.*, 2015)
		Radioprotection	100μM g^{-1} TEAC		
		OH. Assay	308.8±14.08μM g^{-1} BHT eq.		
		β-carotene bleaching	25.11±1.50μM g^{-1} BHT eq.		
		Reducing power (EC_{50})	181.4μg mL^{-1}		
Analgesic and anti-inflammation	*In vivo*	Rats induced CFA-injection	20mg kg^{-1}	γ-Terpinene, linalool, myristicin, safrole, eugenol, and elemicin	(Wahab *et al.*, 2009; Asgarpanah, 2012; de Cássia da Silveira e Sá *et al.*, 2014; Passos *et al.*, 2015)
Anti-convulsant	*In vivo*	Electroshock seizure test in mice	200μL kg^{-1}	Pinene analog, linalool, α-terpineol, eugenol, and citronellol	(Wahab *et al.*, 2009; de Sousa *et al.*, 2010; Bahr *et al.*, 2019)
Anti-infection	*In vitro*	Anti-parasitic assay (EC_{50}) *Toxoplasma gondii*	24.45μg mL^{-1}	Linalool, β-caryophyllene, pinene analogs, p-cymene, and carvacrol	(Dorman and Deans, 2004; Prabuseenivasan *et al.*, 2006; Gupta and Rajpurohit, 2011; Pillai *et al.*, 2012; Soni *et al.*, 2016; Nurjanah *et al.*, 2017; Monzote *et al.*, 2019)
		Intracellular anti-amastigote test (IC_{50}) *Leishmania amazonensis*	133.5μg mL^{-1}		
		Dilution method (MIC)			
		S. aureus	8μL at 12.5% v/v		
		E. coli	12μL at 12.5% v/v		
		P. vulgaris	10μL at 12.5% v/v		
		B. subtilis	10μL at 12.5% v/v		
		K. pneumoniae	8μL at 12.5% v/v		
		B. megaterium	14μL at 12.5% v/v		
		Disk diffution method			
		P. aeruginosa	20-100 % v/v		
		S. epidermis	20-100 % v/v		
		S. dysenteriae,	20-100 % v/v		
		S. typhi	20-100 % v/v		

Antioxidant

The production of reactive oxygen and nitrogen species (ROS/RNS) causes oxidative stress that prompts the development of age-related ailments such as neurodegenerative diseases, kidney disorder, cardiovascular maladies, macular degeneration, biliary diseases, cancer, and chronic obstructive pulmonary disease, as well as sarcopenia and frailty (Huang *et al.*, 2005; Liguori *et al.*, 2018). Antioxidants are compounds that prevent the oxidation process or neutralize free radicals i.e., (ROS/RNS) (Oke *et al.*, 2009; Yashin *et al.*, 2017); therefore, they are potentially used to treat age-related and disease-related oxidative stress (Liguori *et al.*, 2018). Spices and aromatic herbs are rich sources of chemical compounds with antioxidant properties (Yashin *et al.*, 2017), including nutmeg (Dragland *et al.*, 2003).

It was reported that the drying method of nutmeg fruit flesh influenced the NEO quality. The shade-drying method had the best antioxidant activity (72.82%) in comparison with the fresh or without drying (24.18%) and sun-drying (45.56%) methods (Sipahelut *et al.*, 2020). Antioxidant activity test with various methods on NEO reported that NEO had antioxidant value with Trolox equivalents of 2.94±0.09μMg^{-1} [2,2-diphenyl-1-picrylhydrazyl (DPPH) assay], 100μMg^{-1} (radioprotective potential), and 308.8±14.08 μMg^{-1} (OH. assay), as well as 25.11±1.50μMg^{-1} BHT equivalent (β-carotene/linoleic acid assay) (Adiani *et al.*, 2015). Another study reported that NEO had reducing power with an EC_{50} value of 181.4 μg mL^{-1} (Piaru *et al.*, 2012b).

Table Ib. Myristica fragrans Houtt. essential oil bioactivities

Biological activities	Study model	Assay	Concentration or dose	Active constituent of NEO	Ref.
Anticancer	*In vitro*	MTT assay (IC_{50})		Myristicin, limonene, eugenol, and 4-terpineol	(Parija and Das, 2003; Calcabrini *et al.*, 2004; Lee *et al.*, 2005; Jaganathan and Supriyanto, 2012; Piaru *et al.*, 2012a, 2012b)
		HCT-116	78.61μg mL^{-1}		
		MCF-7	66.45μg mL^{-1}		
	Ex vivo	Rat aortic ring assay (IC_{50})	77.64μg mL^{-1}		
Insecticidal	*In vitro*	Intrinsic insecticidal activity (LD_{50})		Eugenol, methyleugenol, methylisoeugenol, elemicin, myristicin, safrole, limonene, α-pinene, β-caryophyllene	(Du *et al.*, 2014; Norris *et al.*, 2015; Soni *et al.*, 2016; Gnankiné and Bassolé, 2017)
		Anopheles gambiae			
		West Indies NEO	10.5μg g^{-1} mosquito		
		East Indies NEO	19.0μg g^{-1} mosquito		
		Aedes aegypti			
		West Indies NEO	19.1μg g^{-1} mosquito		
		East Indies NEO	33.3μg g^{-1} mosquito		
		Fumigant toxicity assay (LC_{50})			
		Plodia interpunctella	6.65μL L^{-1}		
		Intrinsic insecticidal activity (LD_{50})*Lasioderma serricorne*	19.3μg per adult		

Reports on NEO's antioxidant constituents were found in various studies. The antioxidant constituents are including elemicin, 4-terpineol, myristicin, trans-sabinene hydrate (Adiani *et al.*, 2015), safrole, eugenol, β-caryophyllene, and isoeugenol (Gupta and Rajpurohit, 2011). Elemicin was reported to be the most potent antioxidant compound (Adiani *et al.*, 2015). Eugenol and β-caryophyllene have hydrogen atoms in the benzylic and/or allylic positions that could easily interact with peroxyl radicals formed under oxidative stress. Eugenol also stimulates the activities of various enzymes that might be beneficial contributors for antioxidant activity e.g., glutamine transferase, catalase, glutathione peroxidase, superoxide dismutase, and glucose-6-phosphate dehydrogenase enzymes (Gupta and Rajpurohit, 2011).

Analgesic and antiinflammation

Inflammation is a protective measure of the body to remove noxious stimuli, such as infection and tissue injury (Ferrero-Miliani *et al.*, 2006), and to commence the recovery process (de Cássia da Silveira e Sá *et al.*, 2014). Inflammation can be categorized into acute or chronic, depending on whether the duration of the symptom persists, whether short or a prolonged response (de Cássia da Silveira e Sá *et al.*, 2014; Hawiger and Zienkiewicz, 2019). Chronic inflammation is correlated with several chronic human ailments, such as allergy, atherosclerosis, cancer, arthritis, and autoimmune diseases (Medzhitov, 2008). NEO is traditionally used to relieve sprains, rheumatism, paralysis, and as an alternative remedy to ease toothache (Asgarpanah, 2012; Zhang *et al.*, 2016).

NEO was a potential chronic pain reliever since it was reported to be able to alleviate mechanical allodynia, joint swelling, and heat hyper analgesia of rats stimulated by the complete Freund's adjuvant (CFA)-injection. Oral intake of 20 mg kg^{-1} NEO preparation decreases swelling, and pain scores significantly in comparison with the 30 mg kg^{-1} diclofenac treated group. The analgesic and antiinflammation activity of NEO was associated with its capability to inhibit the expression of blood substance P level and COX-2 (Zhang *et al.*, 2016).

Several compounds that are responsible for the analgesic and antiinflammation activity of NEO are γ-terpinene, linalool (De Brito Passos *et al.*, 2015), myristicin (Asgarpanah, 2012), safrole, eugenol, and elemicin (de Cássia da Silveira e Sá *et al.*, 2014). At low doses, γ -Terpinene could exert an antinociceptive effect in capsaicin, formalin, and glutamate tests through the opioid system involvement via K^{+}_{ATP} channels and the cholinergic

system. γ-Terpinene could inhibit pro-inflammatory mediators and affect the neurotransmission pathways at the SNc level (such as substance P or CGRP)]. Safrole has a very weak anti-inflammatory activity with a percentage activity of rat paw edema inhibition of 5% at 50mg kg^{-1} body (Parise-Filho *et al.*, 2011). Eugenol was reported to be able to suppress the production of thromboxane B2, prostaglandin, and leukotriene. *In vivo* experiments revealed that rats which were pretreated with eugenol at 25, 50, and 100mg kg^{-1} doses were observed to be able to reduce the carrageenan-induced paw edema significantly by 28%, 62%, and 78%, respectively (de Cássia da Silveira e Sá *et al.*, 2014). Myristicin anti-inflammation activity was correlated with its aptitude to suppress the production of several inflammatory regulators, such as calcium, NO, IL-6, IL-10, macrophage inflammatory protein (MIP)-1α, MIP-1β, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-inducible protein-10, monocyte, chemotactic protein (MCP)-1, MCP-3, and leukemia inhibitory factor (LIF, a member of IL-6 family) (Narasimhan and Dhake, 2006; Serhan, Chiang and Van Dyke, 2008; Lee and Park, 2011). While elemicin inhibited 5-LOX, was reported as potent proinflammatory leukotrienes against rat basophil leukemia cells (RBL-1 cells) (de Cássia da Silveira e Sá *et al.*, 2014). Other compounds that have been reported to have antiinflammation and analgesic activity are sabinene, α-pinene, 4-terpineol, limonene, α-terpinene) (Andrade *et al.*, 2015; De Brito Passos *et al.*, 2015; Khalilzadeh *et al.*, 2015). α-Pinene reported impeding the production of pro-inflammatory cytokines i.e., IL-1β, TNF-α, and IL-6, in rats prompted for acute pancreatitis (Bae *et al.*, 2012).

Anticonvulsant

Convulsions happen because of the imbalance between excitatory and inhibitory neurotransmitters. NEO was reported to possess anticonvulsant activity in multiple seizure models. In the maximal electroshock seizure test, 200μL/kg NEO was able to give total protection against hind limb tonic extension phase, while it gave dose-related effect in the pentylenetetrazol, bicuculline, and strychnine seizure tests. NEO's anticonvulsant activity was reported to have a rapid onset of action with a short duration of action (Wahab *et al.*, 2009).

Compounds in the NEO that were reported to have an anticonvulsant effect are pinene analog, linalool, α-terpineol (de Sousa *et al.*, 2010), eugenol, and citronellol (Bahr *et al.*, 2019). The pinene analogs are potent acetylcholinesterase inhibitors, that also work as a modulator on $GABA_A$ receptors and increase the postsynaptic GABA-dependent chloride flow (Zamyad *et al.*, 2019). They also reduced the hippocampal nitrite level and striatal content of norepinephrine and dopamine (Felipe *et al.*, 2019). Linalool affects glutamatergic transmission, it significantly reduces potassium-stimulated glutamate release and uptake, but does not obstruct basal glutamate release (Wahab *et al.*, 2009). Eugenol blocks sodium current, which contributes to the modulation of neuronal hyperexcitability (Huang *et al.*, 2012). Yet, the eugenol effect is dose-dependent. Eugenol could have both neuronal repressive and excitant effects. At low concentration (0.5mM), it has an antiepileptic effect, while at higher concentration (2mM), it induces epileptiform activity (Vatanparast *et al.*, 2017). Terpineol had a dose-dependent and reversible anticonvulsant effect. It inhibits the action potential propagation effect of the rat's sciatic nerve (Moreira *et al.*, 2001).

Antiinfection

NEO was reported to have antibacterial, antifungal, and antiparasitic activities (Gupta and Rajpurohit, 2011; Pillai *et al.*, 2012). The *in vitro* antiparasitic test of NEO against *Toxoplasma gondii* showed a promising inhibition activity with EC_{50} of 24.45μg mL^{-1}, which was comparable to inhibition properties of clinically standard drug, clindamycin, which had EC_{50} of 16.57 μg mL^{-1} (Pillai *et al.*, 2012). It also had relatively weak antileishmanial activity against *Leishmania amazonensis* with an IC_{50} of 133.5μg mL^{-1}. Compounds that reportedly had antiparasitic activity were α-pinene, linalool, and β-caryophyllene (Monzote *et al.*, 2019).

NEO showed inhibition activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus megaterium* (Soni *et al.*, 2016), *Pseudomonas aeruginosa* (Prabuseenivasan *et al.*, 2006), *Staphylococcus epidermis*, *Shigella dysenteriae*, and *Salmonella typhi* (Nurjanah *et al.*, 2017). Several compounds were identified to be responsible for the antimicrobial activity, i.e. pinene analogs, p-cymene, carvacrol, and β-caryophyllene (Dorman and Deans, 2004; Gupta and Rajpurohit, 2011). α- and β-pinene showed toxicity against membrane cells (Alma *et al.*, 2004). α-pinene was also found to be an antibiotic resistance modulator for *C. jejuni* that targets antimicrobial efflux systems (Kovač *et al.*, 2015).

Anticancer

NEO also reported had cytotoxic effects against human colorectal carcinoma (HCT-116) and human breast carcinoma (MCF-7) cell lines with IC_{50} values of 78.61 and 66.45μg mL^{-1}, respectively (Piaru *et al.*, 2012a). Additionally, the antiangiogenic activity of NEO was investigated *ex vivo* using rat aortic ring assay and the IC_{50} was reported to be 77.64μg mL^{-1} (Piaru *et al.*, 2012b). Studies reported that several NEO compounds, i.e., myristicin, limonene, eugenol, and 4-terpineol, had an anti-cancer effect. Myristicin induces the activation of specific intracellular death-related pathways, which precedes caspase-3 stimulation and apoptosis cytotoxicity initiation in human neuroblastoma SK-N-SH cells through apoptotic mechanism (Lee *et al.*, 2005). D-limonene was reported to be a potential chemoprevention agent in N-nitrosodiethylamine induced hepatocarcinoma in AKR mice models through its ability to inhibit the overexpression of c-myc oncoprotein (Parija and Ranjan Das, 2003). 4-Terpineol was reported to be able to prompt caspase-dependent apoptosis of human melanoma M14 WT cells and M14 adriamycin-resistant cells (Calcabrini *et al.*, 2004). Eugenol was reported to be able to stimulate apoptosis in osteosarcoma, skin tumors, melanoma, leukemia, gastric and mast cells (Jaganathan and Supriyanto, 2012).

Insecticidal

Essential oils are generally recognized as safe (GRAS) for the environment and human health. Therefore, NEO is a promising, eco-friendly, and safe insect repellent or insecticidal agent. A study in West Indies and East Indies highlighted NEO toxicity against adult female *Anopheles gambiae* at LD_{50} of 10.5 and 19.0μg g^{-1} mosquito, respectively, while they had LD_{50} of 19.1 and 33.3μg g^{-1} mosquito against adult female *Aedes aegypti* (Norris *et al.*, 2015). Another study reported that NEO had LC_{50} of 6.65μL L^{-1} air against *Plodia interpunctella* adults (Soni *et al.*, 2016) and LD_{50} of 19.3μg/adult *Lasioderma serricorne* (Du *et al.*, 2014). Six active compounds i.e., eugenol, methyleugenol, methylisoeugenol, elemicin, myristicin, and safrole, were identified in the study (Du *et al.*, 2014).

NEO compounds, i.e. limonene, eugenol, α-pinene, β-caryophyllene (Gnankiné and Bassolé, 2017), and myristicin (Srivastava *et al.*, 2001) were reported to be responsible for the insecticidal activity. Myristicin was also reported to have LD_{50} of 104mg per larva against *Spilarctia obliqua* and LD_{100} of 25 ppm against *Aedes aegypti* (Srivastava *et al.*, 2001).

Toxicity

Large intake of NEO might result in intoxication with symptoms such as facial flushing, blurred vision, dry mouth, hypertension, tachycardia, nausea, vomiting, feelings of euphoria and unreality, delirium, agitation, abdominal pain, or hallucinations (Stein *et al.*, 2001; Gupta and Rajpurohit, 2011; Ehrenpreis *et al.*, 2014). The LD_{50} of NEO is 2150 μL kg^{-1} (Wahab *et al.*, 2009). NEO toxic effects are attributed to myristicin, safrole, elemicin, 4-terpineol (Stein *et al.*, 2001; Beyer *et al.*, 2006; Muchtaridi *et al.*, 2010).

Elemicin and myristicin were assumed to cause NEO psychoactivity through a metabolic process in the body and converted into amphetamine derivatives (Gupta and Rajpurohit, 2011; Ehrenpreis *et al.*, 2014). Elemicin is metabolized to 3,4,5-trimethoxyamphetamine, while myristicin to 3-methoxy-4 5-methylenedioxy amphetamine (Stein *et al.*, 2001). Myristicin is also a weak monoamine oxidase inhibitor, that is accountable for certain cardiovascular symptoms (Gupta and Rajpurohit, 2011). Myristicin, 4-terpineol, and safrole were also reported to be associated with locomotor activity inhibition in mice (Muchtaridi *et al.*, 2010). Low concentrations of GABA, 4-terpineol, and α-terpineol were reportedly able to potentiate the $GABA_A$ receptor-mediated response such as sedative and anxiolytic effects (Aoshima *et al.*, 2001; Hossain *et al.*, 2002).

CONCLUSION

NEO can be obtained from seed, leaf, or mace of *M. fragrans*. It is rich in terpenes and phenylpropanoids. The analysis of NEO was mostly done using GC, especially GC/MS methods. The main bioactive substances of NEO are pinene derivates, elemicin, 4-terpineol, myristicin, eugenol, and linalool.

NEO has various pharmacological functions including antioxidant, analgesic, antiinflammation, anticonvulsant, antibacterial, antiparasitic, anticancer/chemopreventive, and insecticidal activities. Further studies particularly *in vitro* studies and biological activity tests directing to *in vivo* pharmacokinetic and pharmacodynamic evaluation that lead to the development of new drug entities are still needed to explore NEO's potential as new therapeutic drugs.

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