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Review Article

Profiling of Eugenol Compounds in Piperaceae, Myrtaceae, Lauraceae and Myristicaceae Using Some Analytical Methods

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ABSTRACT

Eugenol compounds contain several functional groups, namely allyl (-CH_2-CH = CH_2), phenol (OH) and methoxy (-OCH_3). These groups allow eugenol to become the basis for the synthesis of various other compounds of higher value, such as isoeugenol and methyl eugenol. Eugenol compounds and their derivatives have numerous benefits in various industries, such as the pharmaceutical, cosmetic and other chemical industries. Metabolite profiling is a technique that analyses all substances detected in the sample used and identifies certain metabolites, however, there has been no review regarding the method of profiling eugenol compounds in various families. For this purpose, literature searches such as PubMed, ScienceDirect, Wiley, Google and other journal publications were conducted. Several studies related to eugenol profiling reported in the Piperaceae, Myrtaceae, Lauraceae and Myristicaceae families found the highest level of eugenol is in the range 83.6%. The methods used for profiling eugenol compounds are reported as thin-layer chromatography (TLC), gas chromatography-mass spectroscopy (GC-MS) and liquid chromatography-mass spectroscopy/mass spectroscopy (LC-MS/MS).
In this review, profiling of eugenol compounds is best performed by sample preparation using distillation techniques followed by GC-MS analysis.

Keywords: Metabolite profiling, Eugenol, GC-MS, Distillation.

Introduction

Metabolite profiling is an analysis of certain metabolite groups that provide information and data that can be integrated based on the metabolites produced in different sample groupings. This method assists in determining which compounds are present in a plant and must also be fast, sensitive, automated and can include multiple metabolites.[1, 2] Metabolite profiling works by tracing all detectable metabolites using a suitable analytical method.[3]
Eugenol, which has the molecular formula $C_{10}H_{12}O_2$, contains several functional groups, namely allyl (-CH_2-CH = CH_2), phenol (-OH) and methoxy (-OCH_3). The presence of these groups allows eugenol to serve as the basis for the synthesis of various other compounds of higher value, such as isoeugenol and methyl eugenol.[4] Eugenol is a clear to pale yellow liquid with a distinctive, refreshing and spicy aroma.[5] Eugenol and various derivative compounds have a strategic role in many industries, such as the pharmaceutical, cosmetic and other chemical industries. These compounds are widely used in the pharmaceutical industry due to its pharmacological activities as analgesics, anti-inflammatory, antimicrobial, antioxidant, antiviral, antifungal, antiseptic, antispasmodic, antiemetic, stimulant and a local anesthetic. In addition, isoeugenol, one of the derivatives of eugenol compounds, can be used as a raw material for antiseptic and analgesic drugs.[5-7] Historically, metabolite analysis could be performed using spectrophotometric instruments to detect single metabolites or separating mixtures of low complexity by chromatography.[8]

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However, during the last decade, several highly sensitive and accurate methods for analysing compounds in complex mixtures have been used. The main techniques utilized for metabolite profiling are gas chromatography-mass spectroscopy (GC-MS), liquid chromatography-mass spectroscopy (LC-MS), liquid chromatography-mass spectroscopy/mass spectroscopy (LC-MS/MS) and capillary electrophoresis/mass spectroscopy (CE/MS). Sample analysis by GC-MS or LC-MS is required to separate and detect all metabolites.[9,10] Due to the complex nature, separation is carried out to facilitate detection of as many metabolites as possible. Special features for identifying all metabolites using mass and retention time.[11-13] The use of this method has been used in various samples, among others, to identify the metabolite compounds of *Vicia* from China, to identify metabolites in bananas, soybeans and the families Piperaceae, Myrtaceae, Lauraceae and Myristicaceae.[14-18]
Several families of various plants grown in Asia are reported to contain eugenol compounds, namely the Piperaceae, Myrtaceae, Lauraceae and Myristicaceae families. Other families have not been further explored regarding their eugenol content. Based on the author's literature search, the Piperaceae family, specifically *Piper betle* L., *Piper crocatum* and *Piper nigrum* L. *alternifolia*, are known to contain eugenol. Part of the Myrtaceae family, *Syzygium aromaticum* (L) Merr & Perry and *Melaleuca alternifolia* are plants known to have eugenol.[19, 20] From the Lauraceae family, *Cinnamomum sintoc* Blume, *Cinnamomum culilawane* Blume and *Cinnamomum cassia* are plants that contain eugenol.[21-23] The nutmeg plant, from the Myristicaceae family, is also known to contain the eugenol compound.[17]
Family Piperaceae has a wide distribution area, especially in tropical and sub-tropical regions. Plants included in the Piperaceae family are aromatic plants which are usually used for medicinal and ornamental purposes. In comparison to other countries, the number of plant species in the Piperaceae family in Indonesia is likely to increase because there are still many unexplored forests. The most popular type of the Piperaceae family is *P. betle*, a plant that has green leaves and stems that are shaped like hearts and spread out during growth. The

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leaves have a distinctive taste and smell. Current research focuses on the evaluation of the antioxidant and antimicrobial properties of betel leaf.[24, 25] *Crocatum piper* also grows as a spread, however, has a purplish-green stem with a shape resembling a leaf with a tapered top. The colour of the upper leaves is grayish-white and the lower leaves are bright red. This leaf has a very bitter taste and distinctive smell. Also, of the Piperaceae family, *P. nigrum* L. grows vines, has single egg-shaped leaves, oblique rounded base of leaves and tapered leaf tips.[26, 27] This plant is widely grown and utilised in Southeast Asia, Indonesia, Malaysia, Thailand, Pakistan, India, Bangladesh, Sri Lanka, the West Indies and Madagascar.[28,29] *P. betle*, *P. crocatum* and *P. nigrum* L., which belong to the Piperaceae plant, are rich in phenolic compounds, especially eugenol. The main compounds found in *P. betle* are eugenol (25.03%), 2,5-dimethylbenzoic acid (12.08%) and decahydro-4a-methyl-1-methylenyl naphthalene (7.18%).[30] The compounds identified in the crocatum piper were eugenol (21.1%), phenol (13.3%), caryophyllene (9.6%) and cavikol (2%).[31] The compounds identified in *P. nigrum* were *β*-caryophyllenen (23.49%), 3-carene (22.20%) and eugenol (4%).[32] Eugenol contained in plants is reported to have biological effects such as antioxidant, antimicrobial and anticancer.[33-39]

Family Myrtaceae is widely distributed in tropical regions and the Australian continent and is widely used in essential oils with medicinal properties and as spices. The most popular species of the family Myrtaceae reported to contain the compound eugenol is *S. aromaticum* (L) Merr & Perry.[19, 40] This plant produces clove oil with a main content of eugenol which acts as a pain reliever and also warms the body. Another plant from the Myrtaceae family containing eugenol compounds, *M. alternifolia*, is widely grown and used in Asia, Indonesia, Malaysia, Pakistan, India, America, Papua New Guinea, Australia and New Zealand. *S. aromaticum* (L) Merr & Perry has the characteristics of single-stemmed and crossed leaves, elongated leaves, pointed tips, tapered base and pinnate leaf bone arrangement, with younger leaves a light green compilation green and green oil. In comparison, *M. alternifolia* has the characteristics of a small tree about 7 m tall with a dense canopy, giving the tree a smooth appearance that contains a lot of oil.[20,40,41]

In the Asian region, *S. aromaticum* (L) Merr & Perry has antimicrobial properties. In addition, in the Americas region, *S. aromaticum* (L) Merr & Perry has been used traditionally to treat food-borne pathogens, viruses and various bacterial infections. *M. alternifolia* is also used as a topical antiseptic due to its antimicrobial properties, especially in the treatment of acne. The main component in *S. aromaticum* (L) Merr & Perry is eugenol. The content of eugenol compounds can reach 70-90% in clove oil.[19, 40]

Family Lauraceae is widespread in Southeast Asia, Indonesia and tropical America. The genus *Cinnamomum* is included in family Lauraceae, which is reported to contain eugenol compounds and aromatic substances used for cooking purposes and as traditional medicine. *C. sintoc* Blume, *C. culilawane* Bl and *C. cassia* are of the Lauraceae family and are reported to contain eugenol. *C. sintoc* Blume is a tree-shaped plant with a height of 39 m which grows mostly in Malaysia and Indonesia. *C. sintoc* Blume is used in traditional medicine as an anti-diarrhea, wound medicine and itchy skin.[21] *C. culilawane* Bl is a type of plant used by local Papuans as traditional medicine. Reported in literature is the use of part of the skin of the plant for bone pain and restoration of stamina.[23] *C. cassia* is widely cultivated in Asia, including Indonesia, and is used as a mixture of spices in ready-to-eat food.[22] *C. sintoc* Blume and *C. cassia* contain 7.64% eugenol, in comparison to *C. culilawane* Bl, which contains 66.23% eugenol compounds.[21-23]

Family Myristicaceae is a widespread tropical plant mostly found in the Asian region. *Myristica fragrans Houtt* is of the Myristicaceae family, which is reported to contain the compound eugenol. *M. fragrans Houtt* is a known spice of economic value, with multiple purposes. Several studies confirm use of *M. fragrans Houtt* as an antioxidant and antibacterial. The benefits of this plant are the activity of chemical components, namely the eugenol compound, which is reported to have high antioxidant activity. The content of eugenol compounds in *M. fragrans Houtt* has been reported as 6.96%.[17]

A review on eugenol profiling technique of the Piperaceae, Myrtaceae, Lauraceae and Myristicaceae families has never been performed. This review will address the techniques of eugenol profiling to identify the technique resulting in higher eugenol level. The selected technique may also assist future researchers in analysis of eugenol compounds from other plants. It is hypothesised that increased research related to various plants containing eugenol can facilitate the discovery of new drug candidates from natural ingredients, given the high antioxidant activity in eugenol compounds.

Eugenol profiling method

Eugenol profiling method starts from plant collection, determination, extraction process and profiling analysis using TLC (thin layer chromatography), GC-MS (gas chromatography-mass spectroscopy and LC-MS/MS (liquid chromatography-mass spectroscopy/mass spectroscopy). TLC is an early stage method as well as simplest method to determine if a sample contains eugenol. A more specific advanced stage is carried out by analysis using GC-MS and LC-MS/MS. Existing research analyses samples of *P. betle* L., *P. crocatum, P. nigrum*, *S. aromaticum* (clove buds and leaves), *M. alternifolia, C. cassia, C. sintoc, C. culilawane* and *M. fragrans Houtt* (root and fruit sections) followed by profiling analysis of eugenol using GC-MS and LC-MS/MS.[17, 19-23, 42, 43]

TLC was used for early detection of a compound, however, according to literature obtained in eugenol analysis, TLC was only used to early detect eugenol in *P. betle, P. crocatum* and *S. aromaticum.* The results obtained from the TLC method used in the *P. betle* and *P. crocatum* samples were prepared by extraction using the maceration method with 96% ethanol solvent while the *S. aromaticum* sample was prepared by the steam distillation method. The results showed that the resulting stain showed an retention factor (Rf) value that referred to the standard used, namely eugenol.[42-44]

The eugenol profiling method in the Piperaceae family in the literature was detected by analysis using GC-MS and LC-MS/MS in comparison to the Myrtaceae family, which was detected by GC-MS analysis. In previous studies the Lauraceae and Myristicaceae families were only detected by analysis using GC-MS, which is expected since eugenol compounds are mostly contained in essential oils in which the analysis of compounds from volatile oil samples was carried out using GC-MS.

Analysis by GC-MS method

The profiling method using GC-MS has a detection sensitivity for almost all volatile chemical compounds.[45] Eugenol compounds are found in essential oils which can be analysed using GC-MS. GC-MS was used in all family plants discussed.

Family piperaceae

Eugenol analysis using the GC-MS method on several types of plants from the Piperaceae family (specifically *P. betle*) was carried out by the extraction process using the maceration method with ethyl acetate as a solvent obtained 25.03% levels at a retention time of 7.714.[30] Treatment with the extraction process using the Simultaneous Distillation-Extraction (SDE) technique obtained 85.18% levels at a 54.16 retention time.[28] In comparison, treatment with the extraction process using the maceration method with 96% ethanol solvent obtained levels of 21.09%, however the retention time is unknown.[42]

Review of past studies of eugenol analysis in *P. crocatum* revealed one article addressing the acquisition of eugenol levels using GC-MS. The treatment was carried out by the extraction process using the digestion method with ethanol as a solvent that is heated at 40-50ºC.

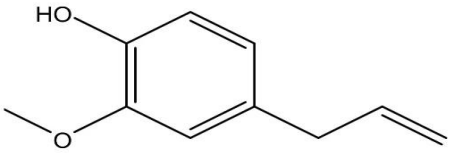

Figure 1: Chemical Compounds of Eugenol

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This method obtained eugenol levels of 21.1-21.9%, but the retention time was not explained in detail.[31] The advantage of the digestion method is that both the solubility and distribution coefficient increase. Its disadvantage is that volatile compounds are continually distilled and heated, thus increases the likelihood of damage that may affect concentration. Past research studies have displayed that the length of time of extraction will affect the percentage of eugenol compounds.

Analysis of eugenol by GC-MS method on *P. nigrum* carried out by the extraction process using Solid Phase Microextraction (SPME) method obtained levels of 7.39% at the retention time of 17.03.[36] The treatment with the extraction process using methanol was identified at 8.523 retention time, without explanation of amount of eugenol content obtained.[32] In the analysis of eugenol in *P. nigrum* conducted by Liu *et al*.[36], the SPME method is one method of extracting samples without solvents, thereby reducing costs, time and pollution that may arise due to the use of solvents. The amount of analyte that can be extracted by fiber in SPME is influenced by various factors including fiber type, temperature and extraction time, stirring, pH and sample volume. The weakness of this method is that optimisation must be carried out in order to obtain the number of compounds with most favorable concentrations. Analysis of eugenol in *P. nigrum* as reported by Mohammed *et al*.[32] can be performed by maceration extraction methods, however since the amount of content contained is not explained in detail it cannot be compared to other methods.

According to previous studies, extraction of Piperaceae plant samples using SDE revealed highest eugenol content. Extraction using SPME was found to produce the lowest eugenol concentration. Maceration with ethanol was utilised in two plants, *P. betle* and *P. crocatum*, and was found to have almost similar eugenol concentrations in the range of 21.09-21.1%. A summary of result of eugenol content found in Piperaceae analysed by GC-MS is displayed in Table 2.

Family myrtaceae

Analysis of eugenol by GC-MS method of several types of Myrtaceae family plants, specifically on the leaves of *S. aromaticum*, was performed by the extraction process using steam and water distillation methods. The eugenol level obtained was 83.6% at 17.596 retention times. The result obtained by evaluation of the seeds using the same extraction process was 74.6% eugenol level at 16.96 retention time.[3] Profiling of eugenol compounds in the genus *S. aromaticum* on the leaves and seeds exemplified that the eugenol content was mostly contained in the leaf part. Analysis of eugenol by GC-MS method of *M. alternifolia* performed by the extraction process using the steam and water distillation method obtained 16.42% methyl eugenol content at 10.29 retention time.[20] Analysis of *S. aromaticum* and *M. alternifolia* shows that there is a similarity in the Myrtaceae family, which contains eugenol compounds. However, there is also a difference, since the content of eugenol compounds with higher levels has been found in *S. aromaticum.*

Family Lauraceae

Eugenol analysis using GC-MS method was performed on several types of Lauraceae family plants, specifically *C. sintoc* Blume, *C. culilawane* BI. and *C. cassia*. Analysis of eugenol in *C. sintoc* Blume was carried out by the extraction process using steam and water distillation methods, resulting in 7.64% of eugenol, compared to *C. culilawane* BI. with a eugenol level of 66.23% using maceration with ethanol followed by distillation to evaporate the solvent.[23] Lastly, eugenol in *C. cassia* was detected at a level of 7.64% using steam distillation for 4 hours.[22] Eugenol profiling in the Lauraceae family shows highest eugenol levels with methods of maceration with ethanol followed by distillation. This result is expected to be due to the hard texture of the stem bark, requiring maceration extraction method initially to extract a large number of metabolites contained therein. A summary on eugenol analysis in family Lauraceae using GC-MS is displayed in Table 4.

Family Myristicaceae

Review of studies of eugenol analysis using the GC-MS method on plant species of the Myristicaceae family was found only in *M. fragrans Houtt*, also known as nutmeg in fruit and roots. In these studies, analyses were accomplished by the extraction process using maceration with methanol solvent, then re-extraction was carried out using the partition method using solvent n-Hexane. The analysis was performed using GC-MS and the eugenol content of n-Hexane nutmeg extract was 6.96% at the retention time of 24.05.[17] Using the same extraction method carried out on the roots of the *M. fragrans Houtt* plant, the eugenol levels were 6.37% at the retention time of 24.10.[17] These results are depicted in Table 5. Unlike other families that contain eugenol compounds, the levels of eugenol in the family Myristicaceae were found to be lower. This is possible because the levels of eugenol compounds in each family can be different and based on the distinct separation method utilised. For example, the distillation method was used in evaluation of the Piperaceae, Myrtaceae and Lauraceae families in contrast to the maceration extraction technique utilised for the Myristicaceae family samples.

Various volatile active compounds have been reported in the literature of the families Piperaceae, Myrtaceae, Lauraceae and Myristicaceae, which vary depending on the location of growth and cultivation, environment and soil.[50]

Table 1: Eugenol Profiling

Sample	Eugenol Profiling Method			Reference
	TLC	GC-MS	LC-MS/MS	
Piperaceae	Verified[a]*	Verified[b]*	Verified[c]*	28[b], 30-32[b], 36[b], 42[a,b], 46[b], 47[c]
Myrtaceae	Not Verified*	Verified[a]*	Not Verified**	19[a], 20[a], 49[a]
Lauraceae	Not Verified**	Verified[a]*	Not Verified**	21[a], 22[a], 23[a]
Myristicaceae	Not Verified**	Verified[a]*	Not Verified**	17[a]

*Verified contains eugenol **Not verified contains eugenol

Table 2: Eugenol Profiling in family Piperaceae by GC-MS Method

Sample	SDE		Maceration Methanol		Maceration Ethanol		Maceration Ethyl Acetate		SPME		Reference
	RT	%	RT	%	RT	%	RT	%	RT	%	
P. betle L.	54.16	85.18[a]	-	-	< 20	21.09[b]	7.71	25.03[c]	-	-	28[a], 42[b], 30[c]
P.crocatum	-	-	-	-	-	21.1	-	-	-	-	31
P. nigrum	-	-	8.52[a]	-	-	-	-	-	17.03	7.39[b]	32[a], 36[b]

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Various methods for the extraction of essential oils from various plants have been carried out in several studies. Maceration is the easiest and fastest method for extracting solvents. The disadvantage of this method is that other non-volatile components can interfere with the analysis using GC-MS. Another method that has been used is the steam-water distillation method. One important factor in extraction is the selection of the method used, resulting in differences in levels of the profile of eugenol compounds.

The extraction method of plants of the family Piperaceae, Myrtaceae, Lauraceae and Myristicaceae revealed SDE as best preparation method before GC-MS to produce the highest eugenol levels. SDE is widely recognized as one of the leading methods for isolating volatile compounds from plant samples.[51, 52] In previous studies, SDE was successfully applied to isolate volatile compounds in betel leaves which were then analysed by GC-MS.

SDE technique is a closed and continuous extraction system, allowing absorption of target components as a whole. In addition, the water temperature circulating in the SDE can reduce the loss of excess volatile compound components in the tested sample so that the results obtained from the analyte can be identified more optimally than the maceration method. Therefore, quantitative data obtained shows the percent area level of a compound identified by the SDE technique is greater than the maceration method.

Analysis by LC-MS/MS Method

The eugenol profile carried out using the GC-MS and LC-MS/MS methods showed that eugenol is a major component in family Piperaceae, especially *P. betle*. In contrast to the analysis using GC-MS, the LC-MS/MS analysis is capable of analysing more compound components.[48] In its treatment, however, the LC-MS/MS method does not have a database reference.

Based on several studies that have been conducted relating to eugenol profiling using GC-MS, identification of eugenol in the families of Piperaceae, Myrtaceae, Lauraceae and Myristicaceae has been successful using various extraction methods. Eugenol compounds are the main components that have been identified as having excellent activity. Eugenol profiles using the LC-MS/MS method also obtained eugenol compounds, however, the percentage obtained was lower than using GC-MS. This can be due to the amount of eugenol in phenolic compounds in essential oil content compared to eugenol in phenolic compounds in non-essential oil content, where volatile essential oils are suitable for analysis using the GC-MS method and by the extraction process using the distillation method.

These findings are consistent with the literature that contend that volatile compounds can be analysed using GC-MS, which is capable of evaluating such compounds. The TLC method may also be used in the initial identification of the eugenol profile.

Table 3: Eugenol Profiling in family Myrtaceae by GC-MS Method

Sample	Method Distillation		Reference
	RT	%	
S. aromaticum (leaf)	17.596	83.6	49
S. aromaticum (bud)	16.96	74.6	19
M. alternifolia	10.29	16.42	20

Table 4: Eugenol Profiling in family Lauraceae by GC-MS Method

Sample	Method Distillation		Method Maceration-Distillation		Reference
	RT	%	RT	%	
C. sintoc Blume	-	7.64	-	-	21
C. culilawane Bl	-	-	12.027	66.23	23
C. cassia	8.958	7.64	-	-	22

Table 5: Eugenol Profiling in family Myristicaceae by GC-MS Method

Sample	Method Maceration n-Hexane		Reference
	RT	%	
M. fragrans Houtt (fruit)	24.05	6.96	13
M. fragrans Houtt (root)	24.10	6.37	13

Table 6: Eugenol Profiling by LC-MS/MS Method

Sample	Method Maceration		Reference
	RT	%	
P. betle	2.102	2.95	47

Table 7: Eugenol Profiling with comparison of GC-MS and LC-MS/MS methods

Family	Sample	Eugenol Profiling (%)		Reference
		GC-MS	LC-MS/MS	
Piperaceae	*P. betle L.* (leaf)	85.18[a]	2.95[b]	30, 31[c], 32, 36, 42, 46, 47[b]
	P. crocatum (leaf)	21.1[c]	-	
	P. nigrum (bud)	4.00[d]	-	
Myrtaceae	*S. aromaticum* (leaf)	83.6[a]	-	49[a], 19[b], 20[c]
	S. aromaticum (bud)	74.6[b]	-	
	M. alternifolia (leaf)	16.42[c]	-	
Lauraceae	*C. sintoc Blume* (wood)	7.64[a]	-	21[a], 23[b], 22[c]
	C. culilawane Bl (wood)	66.23[b]	-	
	C. cassia (wood)	7.64[c]	-	
Myristicaceae	*M. fragrans Houtt* (fruit)	6.96[a]	-	17[a]
	M. fragrans Houtt (root)	6.37[a]	-	

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The analytical methods used each have their own weaknesses and strengths. For example, the GC-MS method and the LC-MS / MS method, however, both methods can produce qualitative and quantitative data. Based on the odorous nature of eugenol compounds, the GCMS analysis method is much more effective for use in various families than other methods. This is also shown at a much greater level of gain in the analysis using GCMS compared to other methods. In comparison, the TLC method can only produce qualitative data.

Conclusion

Eugenol is a component of compounds in the families Piperaceae, Myrtaceae, Lauraceae and Myristicaceae, especially in the family Piperaceae. The choice of method can be adjusted to the needs of the analysis to be carried out, in a qualitative or quantitative form. The analytical method that best produces qualitative and quantitative data for the manufacture of metabolite profiles of the family Piperaceae, Myrtaceae, Lauraceae and Myristicaceae is GC-MS.
The GC-MS method is a better procedure for identifying compounds, especially eugenol compounds in plants belonging to the Piperaceae, Myrtaceae, Lauraceae and Myristicaceae families; therefore, it is hypothesised that this method can be optimised for the metabolite profile of various other plants.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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