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MINI REVIEW

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Estragole and Anethole as Potential Hazardous Compounds in Products: Extraction, Analysis and Pharmacological Effects.

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Introduction

Over the past decade, there is an increase in the usage of herbal products due to their effectiveness, cheapness, and safety compared to chemical drugs which are expensive and have side effects **(Bandaranayake, 2006; Ekor, 2014)**. The potential bioactivity of medicinal plants can be linked to their secondary metabolites. However, the conception of identifying natural products are safe should be reviewed as their role can be reversed to toxic effect (examples: carcinogenic, hepatotoxic and gastrointestinal toxicity) as a result of exposure level to toxic compounds **(Guldiken *et al.*, 2018)**. Volatile oils are among the secondary metabolites present in aromatic herbs. Essential oils are complexes of organic compounds which according to their hydrocarbon Skelton can be divided into two main groups: terpenoids and phenylpropanoids **(Dhifi *et al.*, 2016; Jugreet *et al.*, 2020)**.

Phenylpropanoids can be divided according to the position of double bond to allylbenzenes (2,3-double bond) which are considered as toxic such as estragole and methyl eugenol and propenyl benzene derivatives (1,2-double bond) that are considered as relatively safe such as anethole and isoeugenol **(Ávila *et al.*, 2009; Rietjens *et al.*, 2005)**. For many consumers, to evaluate safety of natural products, there is a need to obtain data about the concentration level of phenylpropanoids in their food. This level can be varied according to many reasons like time of harvesting, part of plant used and the extraction methods **(Eisenreich *et al.*, 2021; Götz *et al.*, 2023)**.

In this review article, estragole and trans-anethole was chosen as compounds of choice for evaluating their presence in plants. This emanated from their double role as the useful bioactive compounds in many edible plants like basil, anise, star anise and fennel and at the same time, their negative impact on various cellular processes. In the following parts, we will briefly summarize the uses of herbs that are the main sources of estragole and trans-anethole besides underscoring their methods of extraction and analysis.

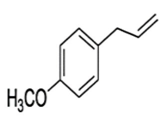

Figure 1. Chemical structure of Estragole

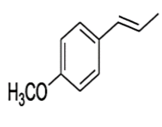

Figure 2. Chemical structure of Trans-anethole

1.1. Chemical nature of estragole and trans-anethole

The presence of estragole and trans-anethole (Table 1, Figure 1, Figure 2), as main components in essential oils of a number of widely used plants like basil, fennel, star anise and anise seeds, can play an important role in their pharmacological activities (Khan and Abourashed, 2011; De Vincenzi et al., 2000). The following (**Table 1**) contain some examples of commonly used herbs to shed more light on presence of estragole and trans-anethole in our daily life.

1.2. Bioactivity and toxicity of estragole and anethole and/or herbs containing them

1.2.1. Traditional and pharmacological uses

The main sources of estragole and trans-anethole are herbs that are commonly used in our daily life. Besides their culinary uses in many food products they are also considered to have many pharmacological benefits. The following table summarize the medicinal and culinary uses of the main sources of estragole and trans-anethole (Table 2)

Table 1. Chemical nature and occurrence of Estragole and trans-anethole

	Estragole	Trans-anethole	Reference
Phenylpropanoids groups	allylbenzene (2,3-double bond)	propenyl benzene (1,2-double bond)	(Eisenreich *et al.*, 2021)
CAS No.	140-67-0	104-46-1	
Molecular formula	$C_{10}H_{12}O$	$C_{10}H_{12}O$	
Molecular weight	148.2	148.2	
IUPAC name	1-allyl-4-methoxybenzene (1-methoxy-4-prop-2-enylbenzene)	4-propenylanisole (1-methoxy-4-(E)-prop-1-enylbenzene)	
Synonyms	Methyl Chavicol	Anise Camphor	
% Occurrence in essential oil			
Fennel	35-58%	4.9-52%	(Shahat *et al.*, 2011; Abdellaoui *et al.*, 2020)
Anise	0.33-5%	82-94%	(Sun *et al.*, 2019; EMA, 2013)
Star anise	Traces-14%	47-90%	(Destro *et al.*, 2019; Sabry *et al.*, 2021)
Basil	2.3-85%		(Padalia *et al.*, 2017; Lee *et al.*, 2005)

Table 2. Traditional and medicinal uses of the known sources of estragole and trans-anethole

plant	Common name	Family	Traditional/ pharmacological uses	References
Foeniculum vulgare **Mill**	Fennel	Apiaceae	• Flavoring agent for baked food • Antibacterial, antioxidant, estrogenic effect • Increase breast milk • Carminative, anticolic, spasmolytic, expectorant	(Rather *et al.*, 2016) (Xu *et al.*, 2020) (ULUSOY *et al.*, 2019) (Noreen *et al.*, 2023; Badgujar *et al.*, 2014)
Pimpinella anisum **L**	Anise	Apiaceae	• Antispasmodic, bronchodilator, expectorant, anti-inflammatory • Antimicrobial, anticonvulsant, estrogenic • Increase milk production	(Sun *et al.*, 2019) (Shojaii and Abdollahi Fard, 2012)
Illicium verum **Hook**	Star anise	Illiciaceae	• Spice in cooking, Antibacterial, antioxidant, antiviral • Antispasmodic, expectorant, carminative, analgesic	(Patra *et al.*, 2020; El-Kersh *et al.*, 2022) (Shahrajabian *et al.*, 2021; Wang *et al.*, 2011)
Ocimum basilicum **L.**	Basil	Lamiaceae	• As raw and cooked form, food production, flavoring agent • Treatment of headache, cold • Antioxidant, antibacterial	(Bakhtiar and Mirjalili, 2024; Li and Chang, 2016) (Ahmed, 2016) (Stanojevic *et al.*, 2017)

1.2.2. Toxicity and scientific opinion

Toxicokinetic and genotoxicity mechanism of estragole is the basis to clarify the argument that estragole is safe or not. European medicine agency EMA has limited the uses of estragole to the lowest level due to their carcinogenicity and genotoxicity **(EMA, 2023)**. On the other hand, Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) has identified estragole as (generally recognized as safe GARS) **(Rietjens *et al.*, 2023)**. The metabolic pathway of estragole and recently trans-anethole was led to formation of DNA adduct which explain the genotoxicity of estragole. However, the possibility of toxicity of anethole was lower than estragole **(Bergau *et al.*, 2021)**. On the same time, anethole was found to have toxicity on pregnant and breast feeding mother due to its estrogenic effect **(Dosoky and Setzer, 2021)**.

1.3. Analysis of estragole and trans-anethole

Recently, the consumption of herbal products has increased. It can be found in many forms as fresh, drink as tea bag form or as ingredient in many food products. As a result, the problem that faced many laboratories that they need a simple, suitable and precise analytical method for identification of herb, determining quality and risk assessment for products. The following table summarize extraction and analytical methods used for detection of estragole and trans-anethole in extract and herbal products of fennel, anise, star anise and basil (Table 3).

Table 3. Summary of extraction procedures and analytical quantification methods of estragole and trans-anethole.

***Foeniculum vulgare* Mill (Fennel)**				
Studied samples	**Extraction method**	**Analytical methods**	**Concentration of analytes**	**References**
Seeds	Hydro-distillation	GC-MS	Estragole (58%) Anethole (5%)	**(Shahat *et al.*, 2011)**
Seeds	Steam distillation	GC-MS	Estragole (35-60%) Anethole (22-52%)	**(Abdellaoui *et al.*, 2020)**
Tea bags	Solvent extraction	GC-MS	Estragole (0.8-4%) Anethole (30-90%)	**(Bilia *et al.*, 2002)**
Herbal products	Hydro-distillation	GC-MS	Estragole (24%) Anethole (40%)	**(Kamal *et al.*, 2017)**
Tea bags, dried seeds	Stir bar extraction	GC-MS	Estragole (9-2056µg/L)	**(Raffo *et al.*, 2011)**
Extract and urine samples	Ionic liquid-based ultrasound assisted surfactant -emulsified microextraction	HPLC	Estragole (12-29µg/ml) Anethole (7-352µg/ml)	**(Rajabi *et al.*, 2014)**
Tea bags, dried seeds	Ultrasound assisted extraction	UPLC	Estragole (0-13000µg/g)	**(van den Berg *et al.*, 2014)**
Seeds, herbal products	Ultrasound assisted extraction	HPTLC	Anethole (8µg/g)	**(Foudah *et al.*, 2020)**
***Pimpinella anisum* L (Anise)**				
Seeds	Hydro-distillation	GC-MS	Estragole (5%) Anethole (87-90%)	**(Anastasopoulou *et al.*, 2020)**
Drinks		HPLC	Anethole (0.1-3g/L)	**(Jurado *et al.*, 2006)**
Seeds	incubation at room temperature	UPLC-MS	Estragole (4422µg/g) Anethole (2312µg/g)	**(AlBalawi *et al.*, 2023)**
Cont. Table. 3				
***Illicium verum* Hook (Star Anise)**				
Commercial samples	Hydro-distillation	GC-MS	Estragole (14%) Anethole (47%)	**(Sabry *et al.*, 2021)**

Fruits	Hydro-distillation	GC-MS	Anethole (97%)	**(Destro *et al.*, 2019)**
Fruits	Steam distillation	HPLC	Anethole (98%)	**(Fagundes *et al.*, 2014)**
***Ocimum basilicum* L. (Basil)**				
Dried Leaves	Hydro-distillation	GC-MS	Estragole (17%)	**(Ahmed *et al.*, 2019)**
Fresh Leaves	Hydro-distillation	GC-MS	Estragole (85%)	**(Padalia *et al.*, 2017)**
Dried leaves	super critical fluid extraction and Hydro-distillation	GC-MS	Estragole (13-18%)	**(Abbas *et al.*, 2017)**
Dried leaves	Hydro-distillation with ultrasound assisted extraction	GC-FID	Estragole (85%)	**(da Silva Moura *et al.*, 2020)**
Dried leaves	Steam distillation	GC-FID	Estragole (2.03mg/g)	**(Lee *et al.*, 2005)**
Dried leaves and stems	Soxhlet extraction	GC-MS	Estragole (39%)	**(Dewi *et al.*, 2023)**
Fresh and urine samples	ionic liquid-based ultrasound assisted surfactant -emulsified microextraction	HPLC	Estragole (0-8.6μg/ml) Anethole (9-505μg/ml)	**(Rajabi *et al.*, 2014)**
Dried Leaves and food product	Ultrasound assisted extraction	HPLC	Estragole (0.7mg/g)	**(Dang and Quirino, 2021)**
Food products	Ultrasound assisted extraction	UPLC	Estragole (2.03mg/g)	**(Al-Malahmeh *et al.*, 2017)**

Conclusion:

Estragole and trans-anethole are the main components of essential oil of various edible herbs extensively consumed in our daily life including basil, fennel, anise and star anise. These herbs have numerous pharmacological and traditional uses. As the uses of these herbs are daily, many studies have been conducted to perform risk assessment of presence of estragole and trans-anethole in herbal extracts, oil and products. The concentration of these compounds in herbal extracts and products varies due to many factors, mainly the methods of extraction and analysis techniques. In conclusion, herbal products should not be overconsumed due to the possibility of high exposure dose of toxic compounds and more studies should conducted to evaluate the safety of these products.

Conflict of interest

The authors declare no conflict of interest

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