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Anethol: A Review

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Abstract:.
Anethols and its derived copmounds are naturally occurring phytochemicals with promising pharmacological properties. These substances used from ancient times throughout the world in traditional medicine. Nowadays it is widely used in food and beverage industry. The present review aims to provide a brief overview of the Physical, Phytochemical, Pharmacological aspects along eith new insights, and upcoming perspectives of Anethol.

Keywords: Anethol, *Foeniculum vulgare*, (E)-1-methoxy-4-(1-propenyl) benzene.

Introduction

Anethole (1-(4"-methoxyphenyl)-prop-1-ene), a pleasant aromatic substance, is present in the oils of a large number of herbs and spices, notably anise. It is consumed in a wide range of foods, ranging from the vegetable fennel, anise and dill flavoured dishes common in Chinese cuisine to aniseed candies and the anise alcoholic beverages (Pastis) beloved in Mediterranean countries.[1] Trans-Anethole (anethole; 1-methoxy-4-(1E)-1-propenylbenzene) is a benzene ring with a single methoxy group para to the double-bonded propenyl group, occurs naturally as a major component of the essential oils in fennel and star anise, and is also present in numerous plants such as dill, basil, and tarragon. The trans isomer is by far more abundant (>99%) than the cis isomer in natural oils.[2] Anethole occurs in nature as both *cis* and *trans* forms, wherein *trans*-isomer being more abundant. *t*-Anethole is a major component of several essential oils, including anise seed oil (80–90%), star

anise oil (>90%) and sweet fennel oil (80%).[3] The primary source of anethole is anise (*Pimpinella anisum*) nonetheless, in Brazil fennel has being the preferred source, instead, due to agricultural difficulties associated with anise cultivation. The major compounds of fennel volatile (essential) oil are trans-anethole (50 to 70%), fenchone (12 to 33%), methyl chavicol (estragole) (2 to 5%); α-pinene, camphene, p-cymene, myrcene, limonene, phellandrene, terpinene, terpineol, cis-ocimene, fenchone.[4] Anethole is industrially utilized as a precursor for 4-methoxyphenyl-2-propanone, a valuable chemical stock. Anethole had been used as the precursor for clandestinely prepared 4- methoxy-amphetamine (PMA) or 4-methoxymethamphetamine (PMMA) through 4-methoxyphenyl-2-propanone. This synthetic route is analogous to the syntheses of the methylenedioxy- amphetamines (MDA, MDMA, or MDEA) from 3, 4-methylenedioxyphenyl-2-propanone, prepared from isosafrole.[5]

Owing to its widespread usage, the potential toxicity of anethole has been studied in vivo and in vitro. from several extensive studies, anethole is considered non-genotoxic and non carcinogenic; it is generally recognized as safe (GRAS) based on the recognized metabolic detoxification of it in human at low levels of exposure (1mg/kg body weight/day) and its low level of use as a flavoring substance (54µg/kg body weight/day), Recently, it has been reported that anethole has anti-oxidant, anti-genotoxic, and anti-carcinogenic activities.[6]

Recently, Shimoni et al.elucidated a *trans*-anethole degradation pathway by Arthrobacter strain TA13 and its mutant strains. Its metabolic derivatives, anisic acid, anisic alcohol, and anisaldehyde, can be also used as a flavoring source for a variety of food additives.[7,8]

Biological Source

Bitter fennel consists of the dry, cremocarps and mericarps of *Foeniculum vulgare* Miller sp. *vulgare* var. *vulgare*. It contains not less than 40 ml/kg of essential oil, calculated with reference to the anhydrous drug. The oil contains not less than 60.0 per cent of anethole and not less than 15.0 per cent of fenchone.[9,10,11]

Anise oil is the common trade name for the essential oils of two different plant species, *Pimpinella anisum* and *Illicium verum*. Most commercially available anise oil is derived from *Illicium verum* (also known as star anise), and is grown primarily in the Far East. Anise oil from *Pimpinella anisum* has a sweeter taste and a more agreeable odor, and is usually grown in Central Asia and the Mediterranean region. The main component of anise oil is anethole,4-methoxyphenyl-1-propene. Both varieties of anise oil contain 80 - 90 % anethole. The essential oil derived from fennel (*Foeniculum vulgare*) also has high anethole content, usually 50 - 60 %.[12,] Commercial sources of anethole include some essential oils (Anise, Star anise,Fennel)[13]

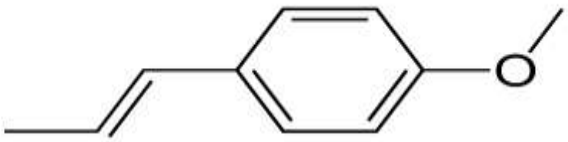
Other Names:[14]

This chemical can be identified by different names, including:

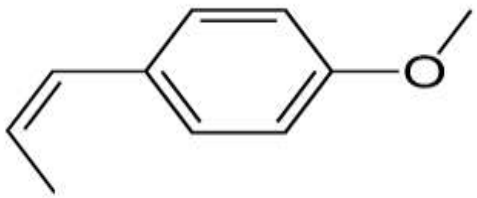
- (E)-1-methoxy-4-(1-propenyl) benzene
- Isoestragole,
- Monasirup
- Trans-1-Methoxy-4-(1-propenyl) benzene
- Trans-Anise camphor
- para-methoxyphenylpropene,
- p-propenylanisole,

Chemistry and Physical Properties of Anethole:

In chemical terms, anethole is an aromatic, unsaturated ether. It has two cis-trans isomers (see also E-Z notation), involving the double bond outside the ring. The more abundant isomer, and the one preferred for use, is the trans or E isomer: trans-anethole, t-anethole, (E)-anethole, trans-para-methoxyphenylpropene. Its full chemical name is trans-1-methoxy-4-(prop-1-enyl)benzene. Anethole occurs in two isomeric forms: (E)-Anethole and (Z)-Anethole.



(E)-Anethole



(Z)-Anethole

Anethole is less soluble in water than in ethanol, which causes certain anise-flavored liqueurs to become opaque when diluted with water (see Ouzo effect). It is a clear, colorless liquid with boiling point 234 °C and congealing point (freezing point) 20 °C;[15] below its congealing point, anethole forms white crystals. The crystals will precipitate from an aqueous solution, which causes a "snow globe" effect when certain anise-flavored liqueurs are chilled. This effect is the basis of a patent for industrial purification of anethole from sources such as pine oil.[16]Anethole can be crystallized directly from a source essential oil by lowering the temperature of the oil; adding a crystal of anethole helps to start the process.[17] Historically, this was used to detect adulteration.[18]

Metabolism of Anethole

The metabolic fate of the naturally occurring food flavouring trans-anethole has been investigated in rats and mice. A single 50-mg/kg dose of trans-[methoxy-^{14}C*]anethole* was given orally to female Wistar albino rats and by ip injection to male CD-1 mice. The major routes of elimination of ^{14}C were the urine and expired air (as $^{14}CO_2$). Excretion of ^{14}C in the faeces and as volatile compounds in the expired air was very low (total < 2 % of the dose). Urinary metabolites were separated by solvent extraction, TLC and HPLC and were characterized by MS and GC-MS directly and following methylation or trimethylsilylation, the results being compared where possible with authentic standards. Eleven ^{14}C -containing urinary metabolites were identified in the rat and ten in the mouse. These compounds arose from side-chain oxidation, side-chain cleavage and various conjugations. The major urinary metabolites were two isomers of 1-(4'-methoxyphenyl) propane-l, 2-diol, 2-hydroxy-l-methylthiol-(4'-methoxyphenyl) propane and 4-methoxyhippuric acid, the first three all being excreted as glucuronides. In addition to these ^{14}C -labelled metabolites, 4-hydroxypropenylbenzene, the unlabelled product of oxidative O-demethylation of trans-[^{14}C]anethole, was excreted extensively in urine as the glucuronide. [19]

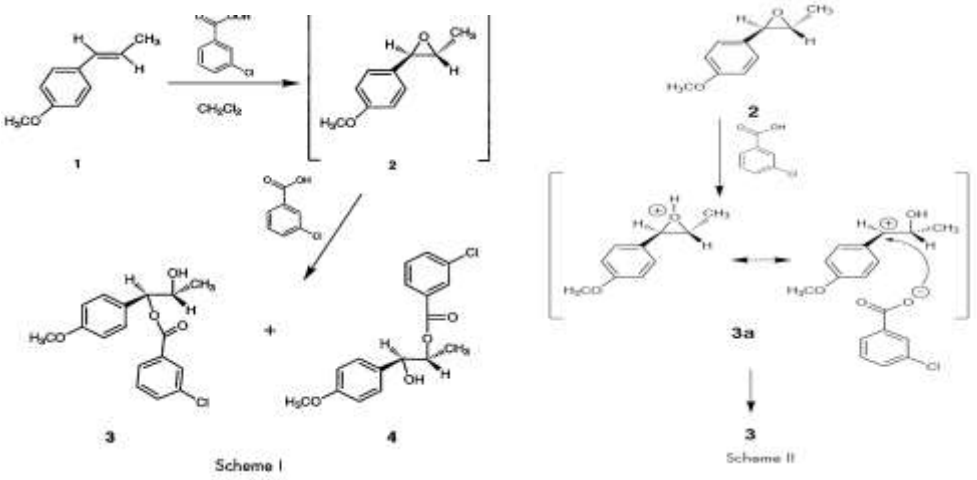
Reactions of Anethole

Epoxidation of Anethole

Epoxidation of alkenes using peroxyacids is one of the most fundamental reactions in organic chemistry, yet there are very few examples of laboratory experiments that illustrate this important reaction. The inherent instability of many epoxides in acidic solutions makes the synthesis of acid-sensitive epoxides by this route

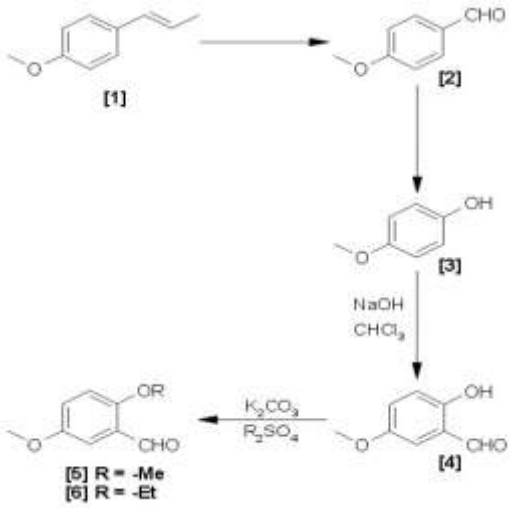
difficult. Frequently, the carboxylic acid formed from the peracid during epoxidation reacts with acid-sensitive epoxides to give α-hydroxyesters as the major product. Procedures have been developed for epoxidation of alkenes in the presence of buffers to minimize this problem.

Scheme -1 and Scheme -2 Represent Epoxidation of Anethole in the absence and in presence of Buffer giving compounds 2, 3, 4.



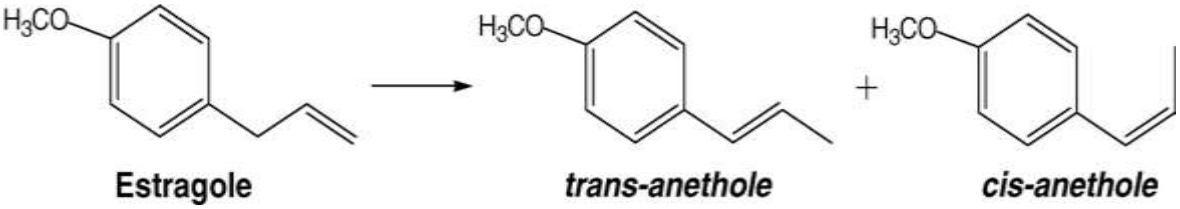
Oxidation of Anethole

Anethole [**1**] is oxidized to anisaldehyde [**2**], which is subjected to a Baeyer-Villiger oxidation to give 4-methoxyphenol [**3**], which is subjected to a Reimer-Tiemann formylation to give 2-hydroxy-5-methoxybenzaldehyde [**4**]. Methylation gives 2, 5-dimethoxybenzaldehyde [**5**], while ethylation gives 2-ethoxy-5-methoxybenzaldehyde [**6**]. Compounds **5** and **6** can be utilized as precursors for various 2, 5-dimethoxylated phenethylamines or 2-ethoxylated-5-methoxylated phenethylamines.



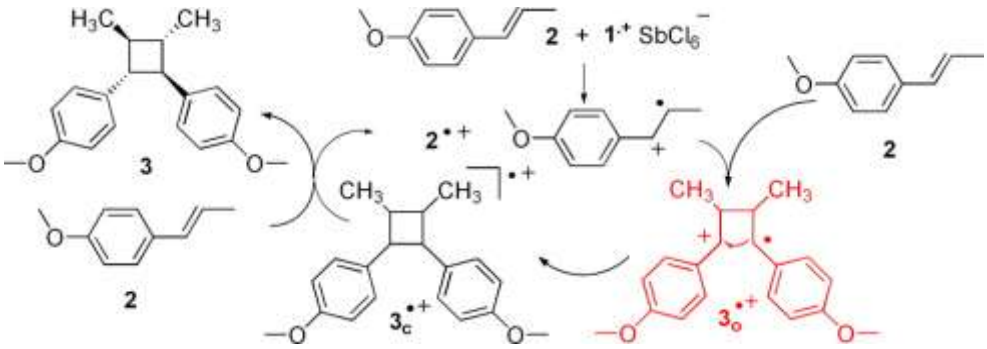
Catalytic isomerization of estragole to anethole[20]

Isomerization of estragole (methyl chavicol) to the corresponding thermodynamically stable anethole (Scheme 1) is an important olefin isomerization reaction wherein the product finds application in alcoholic beverage industry and in oral hygiene products. Anethole occurs in nature as both *cis* and *trans* forms, wherein *trans*-isomer being more abundant. *t*-Anethole is a major component of several essential oils, including anise seed oil (80–90%), star anise oil (>90%) and sweet fennel oil (80%). Industrial sources of anethole have changed to address an increasing demand.



Electron-Transfer-Catalyzed Dimerization of trans-Anethole

The electron-transfer-catalyzed (ETC) dimerization of electronrich styrenes, **2**, has been the most thoroughly investigated ETC [2+2]-cycloaddition. It could proceed stepwise via the formation of a distonic tetramethylene radical cation $\mathbf{3_o}^{\bullet+}$ followed by cyclization to give a cyclobutane radical cation $\mathbf{3_c}^{\bullet+}$ or via a concerted cycloaddition to give in one single reaction step $\mathbf{3_c}^{\bullet+}$ (Scheme 1). In our recent study of the dimerization of *trans*-anethole **2** initiated by tris(4-bromophenyl)-aminium radical cation $\mathbf{1}^{\bullet+}$ using continuous flow and a reaction time of 7 s we could detect only radical cation $\mathbf{3_c}^{\bullet+}$. On the basis of ab initio calculations of the dimerization of 4-methoxystyrene, the two-step mechanism was suggested. However, an unambiguous prediction is not possible on the basis of these results. [21,22,23,24]



A neolignan-type impurity arising from the peracid oxidation reaction of anethole[25]

D. Waumans *et al*.have studied A neolignan-type impurity arising from the peracid oxidation reaction of anethole in the surreptitious synthesis of 4-methoxyamphetamine (PMA) It has been found that 2, 4-dimethyl-3, 5-bis (4'-methoxyphenyl) tetrahydrofuran **(1)**, a chemical substance with a neolignan structure, is formed during the performic and peracetic acid mediated oxidation of anethole. Taking into consideration the manner this impurity is formed during the reaction, it can be argued that this compound is a selective marker for the peracid oxidation reaction of anethole. Its applicability is demonstrated by its presence in clandestinely

manufactured preparations. It should be noted, however, that the presence of this impurity depends on a great deal on the underground chemist's work-up abilities and/or mindset. Since the new impurity is a high-boiling substance, it is unlikely to retrieve it if intermediary purification of PMP2P has occurred.

Anethole as para-methoxyamphetamine (PMA) precursor[26]

D. Waumans et al. synthesized PMA using anethole as starting material. They investigated MDMA is one of the most popular drugs of abuse. Due to its illegality, MDMA and its chemical precursors are watched by governmental organizations in many countries. To avoid conflicts with legal instances, underground chemists have tried to market several new unregulated amphetamine analogues, such as 4-MTA. Para-methoxy amphetamine (PMA), on the other hand, is regulated by law but its precursors are easily obtained since they are cheap and unwatched. This article presents such a case, namely the large scale synthesis of PMA using anethole, a main constituent of anise oil, as precursor. Anethole has been converted to its phenyl acetone analogue via peracid oxidation, while PMA itself has been synthesized using this ketone as precursor in the Leuckart synthesis. The synthesis of PMA using anethole as starting product has been investigated applying GC/ MS and GC-HSPME/MS techniques, hereby discovering new specific (4-methoxyphenol) and already identified synthesis impurities (4-methyl-5-(4 methoxy -phenyl) pyrimidine, N-(b-4-methoxyphenylisopropyl)-4-methoxybenzyl methyl ketimine, 1-(4-methoxy- phenyl)-N-(2-(4-methoxyphenyl)-1-methylethyl-2-propanamine, 1-(4-methoxyphenyl)-N-methyl-N-(2-(4-methoxyphenyl)-1-methylethyl-2-propanamine, N-(b-4 methoxy phenyl- isopropyl)-4-methoxybenzaldimine). The new impurity 4-methoxyphenol is specific for the application of a peracid oxidation method where anethole is used as precursor.

Pharmacological Activities of Anethole:

Antimicrobial Activity[27]

Dorcas Osei-Safo et al, have studied antimicrobial activity of the leaf essential oils of chemo varieties of Clausena anisata (Willd.) Hook. f. ex Benth, The study was carried out as part of this search using the essential oils of the leaves of three chemo-varieties of Clausena anisata (Willd.)

Hook. f. ex Benth. namely, estragole, trans-anethole and feniculin-containing chemo-varieties. The oils were screened against six bacteria (Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shigella sp., Proteus sp. and Pseudomonas aeruginosa) and three fungi (Candida albicans, Aspergillus niger and Aspergillus parasiticus) isolated from clinical specimen using the disc sensitivity test. Microbes which showed significant sensitivity were further assayed with various concentrations of the active extracts in a dilution sensitivity test. The microorganisms were also assayed against seven broad spectrum antibiotics: penicillin G, amoxycillin, ampicillin, tetracycline, ceftizoxime, fosfomycin and urotractin. Results from the disc sensitivity test showed that the estragole-rich oil exhibited significant antimicrobial activity against E. coli (16.3±0.3mm) and Shigella sp. (17.2±0.4 mm). The trans-anethole-rich oil exhibited less significant activity (11.4±0.7mm and 12.1±0.3mm respectively) whereas the feniculin-rich oil, acting alone and in combination with the trans-anethole-rich oil did not show any significant activity against the all microbes tested. Only the neat oils and their 1:2 dilutions showed visible inhibition of microbial growth in the dilution sensitivity test. The estragole-rich oil gave minimum inhibitory concentrations of 3.7, 6.7 and 13.2 mg/ml against C. albicans, S. aureus and E. coli respectively with corresponding ED50 values of 1.3, 2.1 and 1.2 mg/ml. The trans-anethole-rich oil gave a minimum inhibitory concentration of 1.8 mg/ml against C. albicans with an ED50 of 0.2 mg/ml. The findings suggest a significant antimicrobial activity of these plant essential oils though of lower efficacy

compared to ampicillin. The results further suggest that such plant essential oils could potentially be exploited in the development of novel antibiotics.

Cytotoxic and xenoestrogenic effects via biotransformation of trans-anethole on isolated rat hepatocytes and cultured MCF-7 human breast cancer cells[28]

Yoshio Nakagawa, Toshinari Suzuki,reported Cytotoxic and xenoestrogenic effects via biotransformation of trans-anethole on isolated rat hepatocytes and cultured MCF-7 human breast cancer cells. The metabolism and action of trans-anethole (anethole) and the estrogen-like activity of the compound and its metabolites were studied in freshly isolated rat hepatocytes and cultured MCF-7 human breast cancer cells, respectively. The incubation of hepatocytes with anethole (0.25–2.0 mM) caused a concentration- and time-dependent cell death accompanied by losses of cellular ATP and adenine nucleotide pools. Anethole at a weakly toxic level (0.5 mM) was metabolized to 4-methoxycinnamic acid (4MCA), 4-hydroxy-1-propenylbenzene (4OHPB), and the monosulfate conjugate of 4OHPB; the levels of 4OHPB sulfate and 4MCA reached approximately 20 and 200 mM within 2 hr, respectively, whereas that of free unconjugated 4OHPB was less than approximately 0.5 mM. At amoderately toxic concentration (1.0 mM), unconjugated4OHPBreached approximately 10 mM, followed by abrupt loss of 30 phospho- adenosine 50-phosphosulphate (PAPS). Based on cell viability and adenine nucleotide levels, 4OHPB was more toxic than anethole and 4MCA. The addition of 2,6-dichloro-4 nitrophenol (50 mM), an inhibitor of sulfotransferase, enhanced the anethole-induced cytotoxicity associated with losses of ATP, PAPS, and 4OHPB sulfate, and symmetrically increased the unconjugated 4OHPB concentration. 4OHPB as well as diethylstilbestrol (DES) and bisphenol A (BPA), which are known xenoestrogenic compounds, competitively displaced 17b-estradiol bound to the estrogen receptor a in a concentration-dependent manner; IC50 values of these compounds were approximately 1X 10^{-5}, 1X10^{-8} and 5X 10^{-5} M, respectively. 4OHPB also caused a concentration (10^{-8} to 10^{-6} M)-dependent proliferation ofMCF-7 cells, whereas neither anethole nor 4MCA (10^{-9} to 10^{-5} M) affected cell proliferation. However, at higher concentrations (>10^{-4} M), 4OHPB rather than anethole and 4MCAwas cytotoxic. These results suggest that the biotransformation of anethole induces a cytotoxic effect at higher concentrations in rat hepatocytes and an estrogenic effect at lower concentrations in MCF-7 cells based on the concentrations of the hydroxylated intermediate, 4OHPB.

Anethole dithiolethione prevents oxidative damage in glutathione-depleted astrocytes[29]

Benjamin Drukarch et al,studied that Anethole dithiolethione prevents oxidative damage in Glutathione-depleted astrocytes. Astrocytes protect neurons against reactive oxygen species such as hydrogen peroxide, a capacity which reportedly is abolished following loss of the antioxidant glutathione. Anethole dithiolethione, a sulfur-containing compound which is used in humans, is known to increase cellular glutathione levels and thought thereby to protect against oxidative damage. In the present study they found that anethole dithiolethione increased the glutathione content of cultured rat striatal astrocytes. This effect was abolished by coincubation with the glutathione synthesis inhibitor buthionine sulfoximine. Nevertheless, in the presence of buthionine sulfoximine, despite the lack of an increase in the lowered glutathione level, anethole dithiolethione fully protected the astrocytes against the enhanced toxicity of hydrogen peroxide. Thus, apparently other mechanisms than stimulation of glutathione synthesis are involved in the compound's protective action in astrocytes. Considering the occurrence of lowered glutathione levels in neurodegenerative syndromes, they conclude that further evaluation of the therapeutic potential of anethole dithiolethione is warranted.

Anticholinesterase activity of Anethole[30]

Santanu Bhadra et al,studied Anticholinesterase activity of standardized extract of Illicium verum Hook. f. fruits.The aim of this styudy is to evaluate the acetylcholinesterase (AChE) and butyrylcholinesterase inhibitory (BChE) activity of standardized extracts of I. verum and its oil. Present study confirmed that anethole contributed to the anticholinesterase activity of I. verum, with more specificity towards AChE. IC50 for AChE and BChE inhibitory activity of anethole was 39.89±0.32 µg/mL and 75.35±1.47 µg/mL, whereas for the oil, 36.00±0.44 µg/mL and 70.65±0.96 µg/mL respectively. Therefore I. verum can be a good lead as anti-cholinesterase agent from natural resources.

Cholinesterase inhibitory activity by 96 well microtiter plate method:

AChE and BChE inhibitory activity was determined for all the extracts, volatile oil and anethole by using a Bio Rad 96-well microplate reader (680 XR, USA)[31]. Galantamine was used as the standard cholinesterase inhibitor. Principle behind the measurement of cholinesterase is the enzyme, which hydrolyzes the substrate acetylthiocholine/butyrylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which is yellow in colour and detected at 405 nm[32]. 125µL of 3mM, DTNB, 25µL of 15mM ATCI (BTCI when measured the BChE inhibition), 50 µL of buffer and sample was dissolved in phosphate buffer and added in increasing order to each well of 96 well plate and the absorbance was read at 405 nm every 13 s for 65 s. 25 µL of 0.22 U/mL of AChE or BChE was added in each well and the absorbance was measured at every 13 s for 104 s. Each concentration was analyzed for six times. The % inhibition of the enzyme activity due to the presence of increasing test compound concentration was calculated. Inhibition curves were obtained for each compound by plotting the % inhibition Versus the logarithm of inhibitor concentration in the assay solution. The linear regression parameters were determined for each curve and the IC50 values were extrapolated.

Cardiovascular effects of Anethole[33]

Rodrigo Jose´ Bezerra de Siqueira et al,investigated Cardiovascular effects of the essential oil of Croton zehntneri (EOCZ) in conscious rats. In these preparations, intravenous (i.v.) injections of EOCZ (1–20 mg kg^{-1}) and its main constituents anethole and estragole (both at 1–10 mg kg^{-1}) elicited brief and dosedependent hypotension and bradycardia (phase I) that were followed by a significant pressor effect associated with a delayed bradycardia (phase II). The initial hypotension and bradycardia (phase I) of EOCZ were unchanged by atenolol (1.5 mg kg^{-1}, i.v.) or l-NAME (20 mg kg^{-1}, i.v.) pretreatment, but were respectively reversed into pressor and tachycardic effects by methylatropine (1 mg kg^{-1}, i.v.) pretreatment. The subsequent pressor effect and the delayed bradycardia (phase II) remained unaffected by atenolol, but were abolished by l-NAME and methylatropine pretreatment, respectively. In rat endothelium containing aorta preparations, the vasoconstrictor responses to phenylephrine were enhanced and reduced, respectively, by the lower (1–30 µg mL^{-1}) and higher (300–1000 µg mL^{-1}) concentrations of EOCZ. Only the enhancement of phenylephrine-induced contraction was abolished by either the incubation with l-NAME (50 µM) or in the absence of the endothelium. These data show, for the first time, that i.v. administration EOCZ induces an initial hypotension followed by a pressor response, two effects that appear mainly attributed to the actions of anethole and estragole. The EOCZ-induced hypotension (phase I) is mediated by a cholinergic mechanism and seems to result mainly from the

concomitant bradycardia. The pressor response of EOCZ (phase II) seems to be caused by an indirect vasoconstrictive action of EOCZ most likely through inhibition of endothelial nitric oxide production.

Chronic Toxicity/Carcinogenicity Study of Trans-Anethole in Rats[34]

R. Truhaut *et al*. studied chronic toxicity/carcinogenicity study of trans-anethole in rats. A chronic feeding study was carried out in rats with trans-anethole. The test substance was administered in the diet to groups (n = 26-78) of 312 male and 312 female Spraguc-Dawley rats at concentrations of 0, 0.25, 0.5 and 1% for I 17-121 wk. The average intakes of trans-anethole varied from 105-550 mg/kg body weight/day. No apparent treatment-related reactions were noted. The only effect was a transient retardation of body-weight gain. No excess mortality was caused by the treatment. No abnormalities related to treatment were seen on necropsy except for reduced adiposity in the highest dose groups. Haematological assessments did not reveal any changes related to treatment. Histological examination revealed certain non-neoplastic and neoplastic lesions common in older rats. The incidence of some hepatic lesions was significantly higher in some treated groups than in controls: altered cell foci (females of the I% group), nodular hyperplasia (males of the 0.5% group and males and females of the I% groups), benign tumours (females of the I% group) and malignant tumours (females of the 1% group). The results are compared with those of previous investigations. This study stress that the low incidence of hepatocarcinomas is restricted to a single species and sex and to the highest dose tested. This pattern of species, sex and dose dependency strongly suggests that metabolic and pharmacokinetic studies will be helpful in interpreting the significance of the rat tumours with regard to the safe consumption of trans-anethole by man. The changes observed in this chronic feeding study are not thought to be of genetic origin and consequently trans-anethole does not constitute a significant carcinogenic risk to man.

Effects of anethole on the contractility of rat isolated aorta: Involvement of voltage-dependent Ca^{2+}-channels[35]

Pedro Marcos G. Soares et al. Studied Effects of anethole and structural analogues on the contractility of rat isolated aorta: Involvement of voltage-dependent Ca^{2+}-channels. Anethole is a naturally occurring aromatic oxidant, present in a variety of medicinal plant extracts, which is commonly used by the food and beverage industry. Despite its widespread occurrence and commercial use, there is currently little information regarding effects of this compound on the vasculature. Therefore the actions of anethole on the contractility of rat isolated aorta were compared with those of eugenol, and their respective isomeric forms, estragole and isoeugenol. In aortic rings precontracted with phenylephrine (PE; 1 μM), anethole (10^{-6} M– 10^{-4} M) induced contraction in preparations possessing an intact endothelium, but not in endothelium denuded tissues. At higher concentrations (10^{-3} M–10^{-2} M), anethole-induced concentration-dependent and complete relaxation of all precontracted preparations, irrespective of whether the endothelium was intact or not, an action shared by eugenol, estragole and isoeugenol. The contractile and relaxant effects of anethole in PE-precontracted preparations were not altered by L-NAME (10 μM) or indomethacin (10 μM), indicating that neither nitric oxide nor prostaglandins were involved in these actions. The mixed profile of effects was not confined to PE-mediated contraction, since similar responses were obtained to anethole when tissues were precontracted with 25 mM KCl. Anethole and estragole (10^{-6} M– 10^{-4} M), but not eugenol or isoeugenol, increased the basal tonus of endothelium-denuded aortic rings, an action that was abolished by VDCC blockers nifedipine (1 μM) and diltiazem (1 μM), or by withdrawal of extracellular Ca^{2+}. Our data suggest complex effects of anethole on

isolated blood vessels, inducing contraction at lower doses, mediated via opening of voltage-dependent Ca^{2+}-channels, and relaxant effects at higher concentrations that are shared by structural analogues.

Genotoxicity of trans-anethole in vitro[36]

N.J. Gorelick, evaluated previously Trans-anethole genotoxicity both in vitro and in vivo. To ascertain the reproducibility and relevance of previously conducted gene mutation studies, the Salmonella/microsome test and the L5178Y mouse lymphoma TK+/- assay were repeated according to the protocols that previously produced positive results. For the mouse lymphoma TK+/- assay, standard conditions were employed. For the Salmonella/microsome tests, however, metabolic cofactors were supplemented relative to standard protocols. In addition, truns-anethole was evaluated for its ability to induce chromosome aberrations in vitro in Chinese hamster ovary cells. The results presented here indicate that truns-anethole does not increase the mutant frequency in the Salmonella/microsome test, whereas a dose-related response was confirmed in the L5178Y mouse lymphoma TK+/- assay with metabolic activation. The metabolic conditions used in each of the published gene mutation assays may explain the various responses to truns-anethole. Truns-anethole did not induce chromosome aberrations in Chinese hamster ovary cells.

The molecular nature of the genetic change induced in mouse lymphoma cells by truns-anethole has not been identified but the available genotoxicity data are consistent with either a recombination event or a non-DNA reactive mechanism. Considering the truns-anethole genotoxicity data base as a whole, including the positive response observed only in the L5178Y mouse lymphoma TK+/- assay, the irreproducible response in the Salmonella/microsome test, the negative result in the chromosome aberration test in vitro and the results from 32P postlabeling studies in vivo, as well as the occurrence of liver tumors in the rat bioassay only at doses which exceeded the MTD and caused significant liver toxicity, repeated toxic insult followed by compensatory cell proliferation is favored as an underlying mechanism for the observed rat tumorigenic response.

Inhibitory effects of anethole and eugenol on the growth and toxin production of Aspergillus parasiticus[37]

Mehmet Karaplnar studied the antifungal and antiaflatoxigenic activity of anethole and eugenol which are active components of commonly used spices was studied against two strains of *Aspergillus parasiticus.* Anethole, up to concentration of 400 µg/ml where complete inhibition was observed, delayed growth and reduced mycelial weight but it showed a stimulative effect on the toxin production of both strains. At a concentration of 300 µg/ml, eugenol inhibited the growth of both strains; levels of eugenol below 200 µg/ml enhanced production of aflatoxin particularly by *A. parasiticus* NRRL 299.

Inhibitory effect of Anethole on T-lymphocyte proliferation and interleukin-2 production through down-regulation of the NF-AT and AP-1.[38]

Sung Su Yea *et al.* investigated the effect of anethole on T-cell function and the regulatory mechanism of its effect. Direct addition of anethole to B6C3F1 mouse splenocyte cultures produced a concentration-dependent inhibition of the lymphoproliferative response to concanavalin stimulation. Anethole inhibited phorbol 12-myristate 13-acetate (PMA) plus ionomycin (Io)-induced interleukin-2 (IL-2) mRNA expression and protein secretion in EL4 mouse T-cells as determined by quantitative/competitive RT-PCR and ELISA, respectively.To further characterize the mechanism for the transcriptional regulation of IL-2, an electrophoretic

mobility shift assay was performed to evaluate the binding activity of the nuclear factor of activated T-cells (NF-AT), activator protein-1 (AP-1), nuclear factor- κB (NF- κB and octamer binding protein (Oct) in PMA/Io-stimulated EL4 cells. Anethole decreased the NF-AT and AP-1 binding activity, but no signiWcant effect was observed on NF-_B or Oct binding activity. These results suggest that anethole suppress T-cell proliferation and IL-2 production and that the inhibition is mediated, at least in part, through the down-regulation of NF-AT and AP-1.

Potentiation of Fungicidal Activities of trans-Anethole against Saccharomyces cerevisiae under Hypoxic Conditions[39]

Ken-Ichi Fujita and Isao Kubo reported Potentiation of Fungicidal Activities of trans-Anethole against Saccharomyces cerevisiae under Hypoxic Conditions. A naturally occurring phenylpropanoid, trans-anethole (anethole), was assayed for its fungicidal activity in Saccharomyces cerevisiae grown under hypoxic and aerobic conditions. Anethole killed the growing cells in malt extract broth only under hypoxic conditions. Anethole did not exhibit fungicidal effects against non-growing cells. The cells of a mitochondrial-DNA-lacking rho0mutant, which grew fermentatively even in the presence of O2, were killed by anethole regardless of aeration. The fungicidal potency of anethole against a strain capable of respiration under aerobic conditions was enhanced by the addition of a respiratory inhibitor antimycin A3. Therefore, anethole possibly expresses fungicidal activities only against fermentatively growing cells of S. cerevisiae.

Insecticidal Activity of Anethole[40]

Chiou Ling Chang et al,reported Insecticidal Activity of Basil Oil, *trans*-Anethole, Estragole, and Linalool to Adult Fruit Flies of *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae.*In this investigation Basil oil and its three major active constituents (trans-anethole, estragole, and linalool) obtained from basil (*Oscimum basilicum* L.) were tested on three tephritid fruit fly species [*Ceratitis capitata* (Wiedemann), *Bactrocera dorsalis* (Hendel), and *Bactrocera cucurbitae* (Coquillett)] for insecticidal activity. All test chemicals acted fast and showed a steep dose-response relationship. The lethal times for 90% mortality/knockdown (LT_{90}) of the three fly species to 10% of the test chemicals were between 8 and 38 min. The toxic action of basil oil in *C. capitata* occurred significantly faster than in *B. cucurbitae* but slightly faster than in *B. dorsalis*. Estragole acted faster in *B. dorsalis* than in *C. capitata* and *B.cucurbitae*. Linalool action was faster in *B. dorsalis* and *C. capitata* than in *B. cucurbitae*. *trans*-Anethole action was similar to all three species. Methyl eugenol acted faster in *C. capitata* and *B. cucurbitae* than in *B. dorsalis*. When linalool was mixed with cuelure (attractant to *B. cucurbitae* male), its potency to the three fly species decreased as the concentration of cuelure increased. This was due to linalool hydrolysis catalyzed by acetic acid from cuelure degradation, which was confirmed by chemical analysis. When methyl eugenol (*B. dorsalis* male attractant) was mixed with basil oil, trans-anethole, estragole, or linalool, it did not affect the toxicity of basil oil and linalool to *B. dorsalis*, but it did significantly decrease the toxicity of trans-anethole and estragole. Structural similarity between methyl eugenol and trans-anethole and estragole suggests that methyl eugenol might act at a site similar to that of trans-anethole and estragole and serve as an antagonist if an action site exists.Methyl eugenol also may play a physiological role on the toxicity reduction.

Larvicidal and Oviposition-Altering Activity of Trans-Anethole

Mosquitoes are the vectors of important human pathogens, including those responsible for causing malaria, dengue, filariasis and yellow fever.[41] Malaria, with 300–500 million new cases annually and approximately 2.5 million annual deaths, [42]is one of the most devastating diseases affecting humans. *Aedes aegypti* (L.) is the main vector of dengue viruses which cause more human mortality and morbidity than any other arthropod-transmitted viral disease, and rank second only to malaria among mosquito-transmitted infections. An estimated 2.5 billion people are at risk, and over 100 million new cases of dengue occur worldwide each year.[43] Vector control using insecticides has been the primary means of reducing dengue virus transmission,[44] but wide-scale applications of synthetic pesticides can lead to environmental contamination and adverse effects on non-target species, including humans.[45,46] In addition, *Ae. aegypti* has developed resistance to organochlorine, organophosphate, carbamate and pyrethroid insecticides inmany regions of the world,[47] which has hindered control efforts Plant-based chemicals, or phytochemicals, have been used for many years to control insect pests on agricultural crops.[48] Their insecticidal, fungicidal, bactericidal, antiviral, antifeedant or insect growth retardant properties[49] often are the result of synergistic interactions among different biologically active constituents such as terpenoids, alkaloids and phenolics. Most insects treated with essential oils display characteristic neurotoxic symptoms including agitation, hyperactivity, paralysis and quick knockdown.[50] Neem-based products have been used to suppressblood feeding, reduce oviposition and inhibit larval growth in *Culex tarsalis* Coquilett, *Culex quinquefasciatus* Say[51] and *Ae. aegypti*.Marigold extract (an essential oil) is lethal to larvae and adults of *Ae. aegypti* and *Anopheles stephensi* Liston,[52] while some plant essential oils demonstrate oviposition deterrent activities inmosquitoes.[53] In this study, the authors investigated the toxicity of 14 structurally different monoterpenoids, *trans*-anethole and one complex essential oil (rosemary oil from *Rosemarinus officinalis* L., family: Lamiaceae) on first through fourth larval instars of *Ae. Aegypti* with the following specific objectives: (1) to quantify the acute toxicities of these compounds to *Ae. aegypti* larvae; (2) to evaluate the possible synergistic effects of PBO on the larvicidal activity of selected compounds; (3) to evaluate selected compounds for their ability to modify the ovipositional activity of *Ae. Aegypti.* [54]

USES OF ANETHOLE

Anethole (also para-methoxyphenylpropene, p-propenylanisole, and isoestragole) is a phenylpropene, a type of aromatic compound that occurs widely in nature, in essential oils.is a flavoring substance of commercial value. In addition, it is distinctly sweet, measuring 13 times sweeter than sugar. It is perceived as being pleasant to the taste even at higher concentrations. It is unrelated to glycyrrhizic acid, which often co-occurs with it,and also is very sweet. Anethole is used in alcoholic drinks, seasoning and confectionery applications, oral hygiene products, and in small quantities in natural berry flavors.[55] Anethole is an inexpensive chemical precursor for paramethoxyamphetamine (PMA),[56] and is used in its clandestine manufacture.[57] Anethole is present in the essential oil from guarana, which is alleged to have a psychoactive effect; however, the absence of PMA or any other known psychoactive derivative of anethole leads to the conclusion that any purported psychoactive effect of guarana is not due to anethole.[58] Anethole is also present in absinthe, a liquor with a reputation for psychoactive effects; these effects however are attributed to ethanol.[59] Anisyldithiolthione, anethole dithione (ADT), and anethole trithione (ATT), Anise oil, Arq Badi sauf. [60]

Conclusion:

The thorough study and investigation of the available Literature of Anethole clearly shown that, Anethole is naturally occurring pleasant aromatic substance, present in the oils of a large number of herbs and spices, notably anise. It is consumed in a wide range of foods, ranging from the vegetable fennel, anise and dill flavoured dishes common in Chinese cuisine to aniseed candies and the anise alcoholic beverages (Pastis) beloved in Mediterranean countries. Anethole is industrially utilized as a precursor for 4-methoxyphenyl-2-propanone, paramethoxy-amphetamine , a valuable chemical stock.It shows various activities like Antimicrobial, Cytotoxic and xenoestrogenic effects, Anticholinesterase activity, Cardiovascular effects, Chronic Toxicity/Carcinogenicity in Rats, Genotoxicity, Inhibitory effects on the growth and toxin production of Aspergillus parasiticus, Inhibitory effect of on T-lymphocyte proliferation and interleukin-2 production through down-regulation of the NF-AT and AP-1, Fungicidal Activity, Insecticidal Activity, Larvicidal and oviposition-altering activity. Psychoactive effect.

As Anethole and its derivatives has been successfully used in many health problems since a long time, it provides a wide area of interest for the research purposes in development of newer drug molecules. Anethole is a drug of choice for multiple diseases there is need to develope various dosage forms using this naturally occurring Chemical compound.

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