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Estimation of Eugenol in Herbal Plants: A Review

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ABSTRACT
Eugenol, a phytogenic bioactive issue is regularly observed in diverse natural flora owning welldefined useful attributes. Prominent reassets of eugenol are clove, cinnamon, tulsi and pepper. Various extraction strategies had been practiced globally for the extraction of eugenol and different nutraceutics from flora. The maximum drastically hired procedures on this regard consist of solvent extraction, hydrodistillation, microwave-assisted extraction, supercritical carbon dioxide extraction and ultrasound-primarily based totally extraction. Eugenol has been authorised to embody severa useful elements in opposition to a capacious spectrum of existence threatening indispositions together with oxidative stress, inflammation, hyperglycemia, increased ldl cholesterol level, neural issues and cancer. In addition, eugenol has additionally proven robust capability as an antimicrobial agent in opposition to huge levels of pathogenic and spoilage inflicting microorganisms. Predominantly, the precept mechanistic procedures related to the healing capability of eugenol consist of its unfastened radical scavenging activity, obstacle of reactive oxygen species' generation, stopping the manufacturing of reactive nitrogen species, enhancement of cyto-antioxidant capability and disruption of microbial DNA & proteins. Consequently, this newsletter is an try and elucidate the overall properties, reassets, extraction strategies, healing function and related mechanisms of eugenol.

Keywords: Eugenol, Cloves, tree, soaps, detergents etc.

I. INTRODUCTION

The spice called clove is the dried flower bud of the clove tree, Eugenia Caryophylata. Eugenol is derived from the species call Eugenia Caryophyllata which includes excessive stage of eugenol (45-90%). Clove has been utilized in historic China as spice and fragrance. In Chinese conventional medication, clove oil, has been used as carminative, antispasmodic, antibacterial and antiparasitic agent, while, the buds had been used to deal with dyspepsia, acute, persistent gastritis and diarrhea. Several clinical research had been executed on E.Caryophylata oil and its important unstable constituent eugenol, revealing pharmacological homes including anesthetic, analgesic, antimicrobial, antioxidant, antiinflammatory, and anticonvulsant, anticarcinogenic, antimutagenic, repellant and antifumigant activities. Eugenol and its derivatives had been utilized in medication as neighborhood antiseptic and anesthetic and in perfumeries and flavorings. Eugenol is likewise counseled to be a useful antioxidant. In dentistry, it's miles utilized in aggregate with zinc oxide for surgical dressing, brief fillings, and caving liners. Eugenol is likewise utilized in meals enterprise in limited concentrations. FDA has permitted clove oil to be used in meals as a flavoring agent. Eugenol has been categorised as "commonly identified as safe (GRAS)' through the U.S. Food and Drug Administration. However, regardless of good sized use and availability of clove oil, cytotoxicity and genotoxicity research of eugenol is lacking. The intention of this examine is to analyze the cytotoxic and genotoxic results of eugenol. For toxicity V79 cells and Neutral Red Uptake Assay are used as an in vitro cytotoxicity test. Single mobileular Gel Electrophoresis (Comet) assay and Micronucleus assay are used as genotoxicity tests. Genotoxicity research are carried in lymphocytes.

II. THEORETICAL PRINCIPALS

General Properties

Cloves; are the fragrant flower buds of a tree (Syzygium aromaticum) that belongs to the own circle of relatives of Myrtaceae and are normally used as spice. Cloves are harvested

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commonly in Indonesia, India, Madagascar, Zanzibar, Pakistan, Sri Lanka and Tanzania (1).
The clove tree is 8-12 meters tall and has huge leaves and flower buds that have pink colour while they're prepared for collection. The clinical call of clove is Syzygium aromaticum, belonging to the genus Syzygium, tribe Syzygieae, and subfamily Myrtoideae of the own circle of relatives Myrtaceae. Oil of cloves, additionally called clove oil, is an important oil from the clove plant, Syzygium aromaticum (2).

Clove has been used as natural medicine, spice and perfume in China and India for over 2000 years. The medicinal use of clove oil for the remedy of toothache has additionally been endorsed in Europe given that seventeenth century. It has been used as a herbal analgesic and antiseptic in dentistry for its foremost factor eugenol. In the USA clove oil and the primary energetic factor, eugenol, were advised as famous substances of patron products (i.e., soaps, detergents) for the reason that nineteenth century. Clove oil become a endorsed supply to be used withinside the synthesis of vanilla at some stage in the 20 th centuries. Eugenol become remoted from clove oil in 1929 for the primary time, and industrial manufacturing of eugenol started out at some stage in the early Nineteen Forties withinside the United States. Clove cigarettes had been opportunity kinds of tobacco which use together with cigars in 1980s. In 1984 and 1985, americaA Centers for Disease Control acquired eleven case reviews related to the improvement of acute breathing signs due to clove cigarettes (1).
There are 3 kinds of clove oil:

- Bud oil is derived from the flower buds of S. aromaticum. It includes 60–90% eugenol, eugenyl acetate, caryophyllene and different minor constituents.
- Leaf oil is derived from the leaves of S. aromaticum. It includes 82-88% eugenol with very little eugenyl acetate, and different minor constituents.
- Stem oil is derived from the twigs of S. aromaticum. It includes 90–95% eugenol, with different minor constituents (1).

Chemical Structure

Eugenol ($C_{10}H_{12}O_2$or$CH_3C_6H_3$) which is a member of the phenylpropanoid class of chemical compounds is a phenylpropene, an allyl chain-substituted guaiacol. It is a clear to pale yellow oily liquid with the molecular weight of 164.2 g/mol and isextracted from the essential oils especially from clove oil, nutmeg, cinnamon, basil and bay leaf. It has a spicy, clove-like aroma. The name is derived from the scientific name for clove, Eugenia aromaticum or Eugenia caryophyllata. Eugenol is the main component of the essential oil extracted from cloves, comprising 72–90% of the total and is responsible for the aroma of cloves (1).

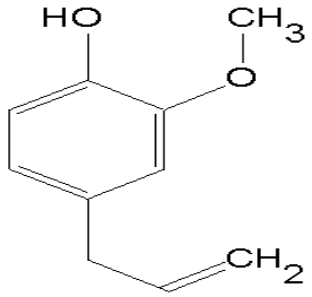

Figure1. Chemical structure of eugenol ($C_{10}H_{12}O_2$)

The synonyms of eugenol are given in the below list:

- Phenol, 4-allyl-2-methoxy
- 4-allylcatechol-2-methyl ether
- P-allylguaiacol
- 4-allylguaiacol
- 4-allyl-1-hydroxy-2-methoxybenzene
- Carophyllic acid
- Eugenic acid
- P-eugenol
- 1, 3, 4-eugenol
- 1-hydroxy-2-methoxy-4-allylbenzene
- 4-hydroxy-3-methoxyallylbenzene
- 1-hydroxy-2-methoxy-4-prop-2-enylbenzene
- 2-methoxy-4-allylphenol,
- 2-methoxy-1-hydroxy-4-allylbenzene
- 2-methoxy-4-prop-2-enylphenol
- 2-methoxy-4(2-propenyl) phenol
- Phenol, 2-methoxy-4-(2-propenyl)

III. EXPERIMENTAL SECTION

Chemicals

Cytochalasin B (Cyt-B)	Sigma
Dimethyl Sulfoxide (DMSO)	Sigma
Ethanol	Sigma
Ethidium Bromide (EtBR)	Sigma

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Ethylene Diamin Tetra Acetic Acid Disodium (EDTA)	Sigma
Eugenol	Sigma
Fetal Calf Serum (FCS),	Biological Industries
Giemsa	Merck
Glacial Acetic Acid	Merck
Heparin (Sodium Salt)	Nevparine®
Histopaque-1077	Sigma
Hydrochloric Acid (37%)	Merck
Hydrogen Peroxide (35%)	Merck
L-Glutamine,	Biological Industries
Low Melting Point Agarose (LMA)	Sigma
Methanol	Sigma
Minimum Essential Medium Eagle (MEM)	Sigma
Neutral Red (NR) Dye	Sigma
Nitric Acid	Sigma
N-Lauryl Sarcosinate sodium Salt	Sigma
Normal Point Melting Agarose (NMA)	Sigma
Penicillin–Streptomycin,	Biological Industries
Phosphate Buffered Saline (PBS)	Sigma
Phytohaemaglutinin-M (PHA-M)	Biological Industries
Potassium Chloride (KCl),	Sigma
Potassium Dihydrogen Phosphate	Sigma
RPMI 1640	Biological Industries
Sodium Chloride (NaCl)	Merck
Sodium Hydrogen Phosphate Dihydrate	Sigma
Sodium Hydroxide (NaOH)	Merck
Tris	Sigma
Triton X-100	Sigma
Trypan Blue	Sigma
Trypsin–EDTA	Biological Industries

Materials and Apparatus

Centrifuge	Heraeus, Hoettich
Cover Slip (24x60mm)	Marienfeld
Deep Freeze (-20oC)	Ariston
Deep Freeze (-800C)	Revco
Electrophorese	Biometra Analitik
Electrophorese Power Supply	Power Pack P 25
Etuve	Dedeoğlu
Fluorescent Microscope	Leica
Incubator	Heraeus Instruments
Inverted Microscope	Leica
Laminar Flow	Heraeus
Magnetic Mixer	Stuart Scientific, 7801Dottingen,M-21
Micro Centrifuge	Heraeus
Micropipettes (1-10 µl, 0, 5-40 µl, 40-200 µl, 200-1000 µl, 1-5ml)	Finnpipette,Gilson, Biohit
Neubauer Slide	Marienfeld
Comet Analysis Software,version 3.0	Perceptives Kinetic Imaging
PH meter	Cyberscan
PH meter Electrode	Sensorel
Scale	Schimadzu Libror
Slides (26x76mm)	Marienfeld

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Spectrophotemeter	Schimadzu Libror
Ultrasonic Bath	Transsonic 460/H
Vortex	Heidolph 2000

Cytotoxicity Assays

The cytotoxicity of eugenol was performed in V79 cells (purchased from Ankara University, Faculty of Pharmacy) by Neutral Red Uptake (NRU) assay following the protocols described by Virgilio et al. (102) and Saquib et al (103).

Solutions of Neutral Red Uptake Assay

1. Eugenol Stock Solution

Eugenol solution is prepared at the concentration of 2 µM. Eugenol is dissolved in distilled water containing 1 % DMSO. Before use, the chemical solution is filtered using Millipore filter.

2. Neutral Red Stock Solution

20 mg of NR powder is dissolved in 5 ml distilled water. The solution must be kept in darkness at 4^0C temperature. The NR Stock Solution can be stored in the dark at 4 ^{0}C for up to one month.

3. Neutral Red Standard Solution

625 µL from stock NR is mixed with 50 ml modified Eagle's medium (MEM). The NR standard solution must be prepared 18 hours before the experiment and must be kept in incubator at 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air.

4. Neutral Red Fixation Solution

A 100 ml of ethanol and 2 ml of acetic acid are mixed with 98 ml distilled water.

Procedure of Neutral Red Uptake Assay

All the method should be completed withinside the laminar go with the drift protection cupboard. The protection cupboard has been very well wiped clean and all system were wiped down with 70% ethanol earlier than use.

1. V79 Cells had been seeded in MEM supplemented with 10�tal calf serum and 1% penicillin streptomycin answer. Cells had been cultured in 25 ml mobileular subculture flasks.
2. Cells had been incubated at 37ºC ± 1ºC, ninety % ± five % humidity, five.0 % ± 1 % CO2/air for twenty-four hours.
three. After 24 hours medium had been aspirated with the aspirator pump. five ml of 370C heat trypsin-EDTA (10X) turned into delivered to the flask to clean the cells, after which the trypsin turned into aspirated. This method turned into repeated once, after trypsinization the cells had been incubated at 37 0C.
4. After incubation, cells had been indifferent from the flask and checked below the microscope to make sure cells being indifferent.
five. 10 ml of 37 0C medium with fetal calf serum turned into delivered to the flasks to prevent the reaction.
6. Cells had been centrifuged for five min at 1200 rpm. The supernatant turned into discarded through aspiration. Cells had been suspended in 2 ml of subculture medium.
7. ninety µL of cells had been combined with 10 µL of trypan blue and the cells had been counted the usage of Neubauer slide.
8. ten thousand cells/ properly in 2 hundred µL medium had been seeded in a 96-properly plate. Each plate turned into managed below a section comparison microscope.
9. Plates had been incubated at 37 0C for 24hours in a humidified surroundings of five% CO2 in air.
10. After 24 hours, the viability and additionally the infection of cells cultured in plates are managed microscopically. Typical symptoms and symptoms of infection are modifications in colour or clouding of mobileular medium and modifications in mobileular shape.
11. Medium turned into discarded from the plate. The cells had been cultured with distinctive concentrations of eugenol for an extra 24 hours.
12. Plates had been incubated at 37 0C for about 18 hours in a humidified surroundings of five% CO2 in air.
13. At the stop of the incubation, the answer turned into discarded from the plates and 2 hundred µl of NR widespread answer at 37 0Cwas delivered to all wells through a multi- channel pipette. The plates had been incubated in 37 0C for extra three hours in a humidified surroundings of five% CO2 in air.
14. At the stop of the incubation, the answer turned into cautiously discarded and plate turned into washed 5 instances with pre-warmed (370C) sterile PBS below the protection cupboard. For washing method, 2 hundred µL of the PBS turned into delivered to every properly through a multichannel pipette after which discarded. Each plate firmly turned into tapped on absorbent paper material to eliminate any final liquid from the wells.
15. 200µl of NR fixative answer turned into delivered to every properly through a multichannel pipette.

16. Plates had been located on shaker for 20 mins at six hundred rpm. Plates had been wrapped in foil to be saved withinside the dark.
17. Plate lids had been eliminated simply earlier than putting every plate at the plate reader.
18. The absorbance of the samples turned into recorded at 540 nm wavelength spectro foto metrically.

IV. CONCLUSION

This review article explicates the effectiveness of eugenol as a therapeutic tool that can be incorporated to various foods and herbal medicines for contending considerable metabolic disorders. It also contains considerable antimicrobial properties and can be employed to inhibit the growth of microbial populations in many foods. Additionally, derivatives of eugenol have unlocked a new era in the domain of pharmacology, kindling the research interests on this compound. Nevertheless, more studies are required to specify the dosage level of eugenol. Eugenol, a major volatile constituent of clove essential oil, is derived from the Eugenia Caryophyllata. Its pharmacological properties which include antimicrobial, anti-inflammatory, analgesic, antioxidant and anticancer activities have been the subjects of many studies.

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