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Percentage and Estimation of Eugenol in Various Herbal Plants

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Abstract
Eugenol, a phytogenic bioactive component is frequently found in diversified herbal plants possessing welldefined functional attributes. Prominent sources of eugenol are clove, cinnamon, tulsi and pepper. Various extraction methods have been practiced globally for the extraction of eugenol and other nutraceutics from plants. The most extensively employed approaches in this regard include solvent extraction, hydrodistillation, microwave-assisted extraction, supercritical carbon dioxide extraction and ultrasound-based extraction. Eugenol has been approved to encompass numerous beneficial aspects against a capacious spectrum of life threatening indispositions including oxidative stress, inflammation, hyperglycemia, elevated cholesterol level, neural disorders and cancer. In addition, eugenol has also shown strong potential as an antimicrobial agent against wide ranges of pathogenic and spoilage causing microorganisms. Predominantly, the principle mechanistic approaches associated with the therapeutic potential of eugenol include its free radical scavenging activity, hindrance of reactive oxygen species' generation, preventing the production of reactive nitrogen species, enhancement of cyto-antioxidant potential and disruption of microbial DNA & proteins. Consequently, this article is an attempt to elucidate the general properties, sources, extraction methods, therapeutic role and associated mechanisms of eugenol

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

Introduction

Herbal formulation

"Crude drugs of vegetable origin utilized for the treatment of disease state of chromic nature. "Herbal medicines includes herbs, herbals materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations. Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities. A holistic approach to herbal medicines, which are as old humankind and contemporary as the latest scientific discoveries in Biomedicine, needs to take into consideration and examine a wide spectrum of sectors, issue and activities including the following:

1. Sources of medicinal plants
2. Sources of information on medicinal plants.
3. Identification, cultivation, harvesting, storage and international trade of medicinal plants.
4. Conservation of biodiversity.
5. Research and development.
6. Intellectual property rights.
7. Production processes- conversion to traditional and modern dosage forms.
8. Safety, efficacy, quality, marketing and sales of herbal medicines.
9. The rational and economic use to herbal medicines.
10. The place of herbal medicines in health care.
11. Training and registration of traditional healers.

A holistic approach to herbal medicines underscores the importance of reorganizing and addressing the needs of each of the different stakeholders and sectors involved. Quite often

each stakeholder examines a particular sector in isolation ignoring the ultimate objective. Consumers on the other hand, are concerned with each and every single sector/issue/activity in this long and complex chain. Herbal medicines, as defined by the WHO, can be classified into three categories;

i. Phytomedicines or phyto-pharmaceuticals sold as over the counter (OTC) products in modern dosage forms such as tablets, capsules and liquids for oral use.
ii. Dietary supplements containing herbal products, also called neutraceutical available in modern dosage forms. Consumers in developed counties and those in urban areas of developing countries use these two types of herbal medicines. These herbal medicines are gradually occupying increasing shelve space in modern pharmacies.
iii. Herbal medicines consisting of crude, semi-processed or processed medicinal plants.

In addition to meeting the health care needs of a vast majority of people and providing the resource base for over half of our modern pharmaceuticals, some medicinal plant based product, though they have not become therapeutically useful drugs, have been instrumental as pharmacological tools to evaluate physiological and patho-physiological processes.

The following examples illustrate how plant based products have been helped scientists to elucidate our understanding in key areas of physiology and pharmacology [1, 2]:

a) The cholinergic system: atropine, muscarine, physostigmine, pilocarpine.
b) The adrenergic system: ephedrine, reserpine, ergot alkaloids.
c) The ganglionic junction: nicotine, lobeline.
d) The neuromuscular junction: tubocurarine.
e) The cardiovascular system: cardiac glycosides, veratrum alkaloids**.**

Result & Discussion

Table 1. Relationship between Concentration Vs. Absorbance And Absorptive.

Concentration (µg/mL)	Absorbance	Absorpitivity
0	0.000	0.0000
10	0.278	0.0278
20	0.574	0.0287
30	0.924	0.0308
40	1.179	0.0294
50	1.550	0.0310
60	1.762	0.0293

Table 2. Statistical data and optical characteristics

Parameter	Values
Max	457
Beer's law	10-60:g
Average mean	0.895
Coefficient of variation	0.651
Standard deviation	0.603
Regression equitation	
Slope	0.0301
Intercept	-0.0085
Coefficient of correlation	0.9977

An excellent correlation was found between absorbance and concentration with value of r=0.9977, intercept value-0.0085 and slope was found to be 0.0301. The equation followed beer's lawin range of 10-60µg/mL. When the marketed formulation containing Eugenol were analysed by this proposed method. The amount of Eugenol was found from 93.16 to 96.40%.

Table 3. Analysis data of herbal formulation

Sample	Label chain (%)	Amount found (%)
Formulation-I	Not less than 85%	94.24
Formulation-II	Not less than 85%	94.40
Formulation-III	Not less than 85%	93.16

When the recovery studies carried out the result was found to be positive.

Table 4. Data of recovery studies

Formulation	Percentage recovery
I	99.90
II	99.80
III	99.20

At last the proposed method was found to be simple, selective and economical; it is cheap as compared to other methods, such as HPLC, GC, etc**.**

Discussion

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. Since the twentieth century, attention has been devoted to the ant mutagenic and genotoxic effects of natural compounds and in recent years there has been a great concern about the activity and the toxicity of eugenol due to its wide range of usage, as well as its antioxidant activity due to the presence of its phenolic group. Eugenol has been classified as generally recognized as safe (GRAS) by the U.S Food and Drug Administration. However, its cytotoxicity and genotoxicity studies are very limited and contradictory. The cytotoxic properties of eugenol have been investigated in different cell lines. The cytotoxicity of eugenol has been studied in three different human derived cell lines i.e. malignant Hep G2 hepatoma cells, malignant Caco-2 colon cells and nonmalignant human VH10 fibroblasts. Eugenol showed cytotoxic effect in all cell lines and acted as a genotoxicant in human VH10 fibroblasts and Caco-2 colon cells but not in Hep G2 hepatoma cells. Eugenol at concentrations below 600 µM significantly increased the level of DNA breaks in human VH10 fibroblast cells and to a lower degree in Caco-2 colon cells. The DNA damaging effect was not observed in Hep G2 cells (73).The cytotoxic effect of eugenol was also investigated in human osteoblastic cell line. Eugenol showed a cytotoxic effect in

human osteoblastic cell line in a dose-dependent manner. The IC_{50} of eugenol in this study was approximately 0.75 mmol/L. Eugenol also inhibited cell proliferation during a 4- day culture period. At the concentrations higher than 0.01 mmol/L eugenol seemed to have significant toxicity potential (69). Yoo CB and et al (59) examined the cytotoxicity of eugenol by using MTT assay in HL-60 cancer cells. Eugenol showed different degrees of cytotoxicity in these cells and it inhibited 50% cell growth in HL- 60 cells at the concentration of 23.7 µM. Another study investigated the effects of eugenol on the growth of human colon cancer cells. HT-29 cells were treated with various concentrations of eugenol (0-250 µM). Eugenol inhibited the growth of cells in a dose and time dependent manner. After 24h exposure, the growth of cells was reduced below 50% at the 250 µM concentrations of eugenol (74). Martins et al (106) examined the genotoxic and apoptotic activities of eugenol in AA8 and EM9 cells. Dose dependent decreases in viability were observed. For a 24h exposure, the cell viability was reduced below 50 % when cells were treated with concentrations higher than 500 µM for AA8 cells and 1000 µM for EM9 cells. The ability of eugenol to induce DNA damage was assessed with alkaline comet assay. In AA8 cells, DNA damage was induced by eugenol, but with no statistical significance. In EM9 cells, eugenol did not induce DNA damage. Studies demonstrated that all the zinc-oxide eugenol based root canal sealers have moderate to severe cytotoxic effects in V79 cultured cells but such effects are different due to the dose and duration of exposure. However, the results did not indicate the genotoxic effects of these dental products (79). In this study we investigated the in vitro cytotoxicity of eugenol by the NRU test in V79 cell line which is widely used healthy cell line in many in vitro assays. Our results demonstrated that the concentrations of eugenol up to 250 µM did not affect the viability of V79 cells during 18 hours exposure, but above this concentration the cytotoxicity of eugenol was observed and the cell viability decreased below 50% at the concentration of approximately 341.5 µM. The data of our study is consistent with the data of Martins et al (106) that indicated cytotoxicity of eugenol at high concentrations, although the IC_{50} value determined in this study is lower than our finding. IC50 values of eugenol have been found to be different according to the cell-line, duration of incubation and the method used in different studies. In these studies, generally cancer cell-lines were used however V79 were used in our study. The genotoxicity of eugenol in V79 cells was investigated in vitro. Eugenol was found to increase chromosomal aberrations with significant increases (3.5% aberrant cells) at 2500 µM, demonstrating cytotoxicity at higher doses (80). A dose and time dependent study in rats investigated the genotoxicity of methyleugenol (MEG) by using comet assay. Results demonstrated no significant differences in DNA damage after 24 hours exposure with doses that produce tumors in rodents (107). In our study, the genotoxicity potential of eugenol was investigated by the alkaline comet assay, a commonly used assay, and the cytokinesis-blocked micronucleus assay (CBMN), at non-cytotoxic concentrations (50-250 µM).

Furthermore, the MN and comet assays were performed to investigate whether eugenol provided protection against H_2O_2 induced DNA damage in human peripheral lymphocytes. H_2O_2 is a highly reactive oxygen species and is able to induce damage to cell membranes, proteins, nucleic acids. It is known to cause oxidative DNA damage primarily through the hydroxyl radical which results from the Fenton reaction. H2O2 has been reported to cause DNA damage in the form of chromosomal aberrations, single and double strand breaks (108). The genotoxic effect of eugenol was investigated by Comet assay in the range of 50-250 µM concentrations. However, only 250 µM eugenol indicated genotoxic effect based on DNA tail intensity data. The same effect was not observed with tail moment and tail length. At the concentrations of 50, 100 µM eugenol, no decrease in the H_2O_2- induced DNA damage was seen. When eugenol used in combination with H_2O_2, eugenol appeared to prevent H_2O_2-induced DNA damage only at 150 µM concentration according to DNA tail length, tail intensity and tail moment data. Eugenol alone, in all study concentrations did not induce any increase in MN. On the other hand, eugenol, in all concentrations, decreased H_2O_2-induced DNA damage. According to this data, it seems that eugenol prevents H_2O_2-induced DNA damage in all study concentrations. In conclusion, the results of this study suggest that eugenol might have cytotoxic effects in a dose dependent manner. However, eugenol in the concentrations used below the IC_{50} values showed no significant genotoxic effects. Our results of MN assay also showed that eugenol might protect against H_2O_2-induced genotoxicity. As our study is composed only an in vitro experiments, further in vivo animal studies are required to understand the genotoxic and antigenotoxic properties of eugenol in detail.

Conclusion

Herbal formulations are prepared from the plant source and the activity of these formulations is mainly due to the active constituent present in them. There are number of herbal formulation available in market. But very few methds have been reported for the quantitative determination of active constituents present in them. Clove is the herbal drug mainly used in dental analgesic, carminative, stimulant, aromatic flavoring agent and antiseptic agent. These actions are due to its active constituents. Which is found in its volatile oil content known as clove oil? The main active constituent is Eugenol (60-90%).

There are two main factors in standardization of herbal formulation are

1. Analyze all herbal ingredients before use in herbal formulation.
2. Develop analytical method for standardize your herbal formulation**.**

The technological advances, commercial factor, changing life style has influenced the way of herbal formulations are being manufactured. There are some common tests used for herbal analysis such as loss on drying, extractive values, ash values, moisture values, microbial contamination and optical rotation.

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