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TO STUDY THE MEDICINAL VALUE OF CLOVE ESSENTIAL OIL

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ABSTRACT
Clove essential oil is valued for its variety of biologically active components and is widely used in the fields of food and pharmaceuticals. Eugenol is the main component that causes the oil's effectiveness. It is an aromatic plant widely cultivated in tropical and subtropical countries, rich in volatile compounds and antioxidants such as eugenol, β-caryophyllene, and α-humulene. Clove essential oil has valued excellent interest due to its wide application in the perfume, cosmetic, health, medical, flavoring, and food industries. Clove essential oil has biological activity relevant to human health, including antimicrobial, antioxidant, and insecticidal activity. Eugenol is the major compound, accounting for at least 50%. The remaining 10–40% contains of eugenol acetate, β-caryophyllene, and α-humulene. In this paper clove oil extracted by hydrodistillation method in lab and found its chemical constituent present in this extracted oil by GC/MS analysis.

Keywords:
Clove essential oil, Chemical biological activity, Extraction, Constituent

1. INTRODUCTION:

Clove is the common name of Syzygium aromaticum or Eugenia caryophyllata, which belongs to the Myrtaceae family. It grows originally on Moluccas Island, Indonesia, but has recently been cultivated worldwide, including Thailand [1]. The dry clove bud is one of the most important aromatic herbs, used as a food additive, flavor, fragrance, and traditional medicine to treat toothache, gastritis, dyspepsia, skin infections, relieve pain, and diarrhea [2]. Up to 15–20 % of the weight of a clove bud is essential oil. This oil contains major components of phenylpropanoids (eugenol and eugenyl acetate), with at least 50 % being eugenol, and also includes minor components of sesquiterpenoids like β-caryophyllene, α-humulene, and others. Clove essential oil, mostly extracted from clove bud, is a clear, pale yellow liquid with a clove- like flavor and aroma. It is well-known and widely utilized in the medical, pharmaceutical, dental care, cosmetic, and food industries. The Clove essential oil a broad range of applications due to its diverse biological functions, including antioxidant, anti-inflammatory, analgesic, antiseptic, anticancer, antibacterial, antifungal, antiviral, and insecticidal properties [3].

The biological activity of Clove essential oil results from its high content of bioactive substances like eugenol. Eugenol has a phenolic group that is of interest due to its strong antioxidant capacity as well as antimicrobial activities, including against food pathogenic bacteria [4]. Additionally, the Food and Agriculture Organization and World Health Organization (WHO) classify eugenol and Clove essential oil (generally recognized as SAF) and non-mutagenic compounds. The maximum daily limit for humans is 2.5-6 mg/kg of body weight [5].

1.1 Extraction of essential oil:

Eos are extracted from plant feedstock by conventional methods, including cold pressing, Hydrodistillation and steam distillation. Additionally, we can include innovative techniques such as Microwave-assisted hydrodistillation, Microwave-assisted steam distillation, and hydro distillation assisted by Ohmic heating [6,7]. In addition, the method used for organic extracts including ultra-assisted extraction (UAE), solvent extraction (SO) and supercritical fluid (SFE), microwave-assisted extraction, ohmic heating-assisted extraction these extracts have a volatile fraction, which is some times erroneously called essential oil [8,9]. There are various methods for the extraction oil. Following methods are-

1.1.1. Conventional / Classical Methods:

The conventional extraction methods are based on the distillation process by heating a plant matrix to recover EO. The extraction is done by injecting steam or water, which crosses the plant matter from the bottom up and carries the volatile materials together with the water as if they were a single component. EO is immiscible in water, making it easily removable by decanting [10]. The HD and SD methods are the best extensively used for extracting Eos. These are easy to operate, have a high reproducibility and do not use organic solvents [10,6,11]

1.1.2. Advanced/Innovative Extraction Methods:

Advanced extraction method, including microwave-assisted extraction (UAE), subcritical fluid extraction (SFE), improve extraction performance, reduce extraction time and energy consumption to obtained organic extracts [12-14]. These methods improve organic compound extraction yield by applying microwave or ultrasonic energy, which can destroy the cell wall of the plant matrix, allowing the compounds to flow better from the biological materials, [8,15,16]. Kennouche *et al*. were reported that the essential oil of Eugenia caryophyllata seed obtained

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by MAE and MSD contained a high percentage of eugenol (65-71%). These extracts preserved their antimicrobial and antioxidant properties.

1.2. Clove Essential Oil Composition (CEO):

At least thirteen (13) compounds have been identified in CEO; Eugenol is the major compounds for at least 78%. The remaining, alpha-caryophyllene is 14%, Eucalyptol 3.96%, cis-bisabolene is 1.2%, Benzyl alcohol is 0.6%, caryophyllene oxide is 0.3%, Limonene is 0.198%, and furfural is 0.06% and traces compounds are 3-allyl6-methoxy phenol, bicyclodecene, methyl benzoate and tert .butyl batene.

1.2.1 Eugenol:

Eugenol is a phenylpropanoid compound found in S. aromaticum L., Cinnamomum spp; P. nigrum, zingiber officinale, organum vulgaris, and T. vulgaris [17]. Eugenol is a volatile compound that varies from colorless to light yellow and has low water solubility (approximately 2460mg/L at 25 degree Celsius), a strong odour and intense flavor. Among the reported biological activities of eugenol are insecticids, antimicrobial, anti-inflammatory, wound healing, antiviral, antioxidant, and anticancer activity [17,20,21,22,23]. It is observed the ant-inflammatory and wound healing ability of a clove oil emulsion in murine experiments. Eugenyl treated skin showed re-epithelialization 20 days after the bound this result was similar to that of a diclofenac gel and a neomycin cream currently used to control inflammation and heal wounds [23]. Eugenol has shown a potential anticancer activity against colon, gastric, prostate, and Lukemia [17]. Eugenol inhibits tumor proliferation and formation increases reactive oxygen species (ROS), generates apoptosis, and has a gentoxic effect in different cancer cells [17,24,25,26].

1.2.2. Alpha-caryophyllen/ Humulene:

A-caryophyllen/Humulene is a sesquiterpene found in S. aromaticum L., senecio brasilinesis humulus lupulus L., and saliva officinalisL. This compound has shown anti-inflammatory and antitumor activity in lung, colon cancer, prostate, and breast cancer. Some studied reported that alpha Humulene demonstrated antiproliferative activity and alteration of the mitochondrial cell membrane in colon cancer cells [19,27,28,29,30,31,32]. It can also improve the antiproliferative effect of cytostatic drugs and other anticancer bioactivities [28,30].

1.2.3. Eucalyptol:

Eucalyptol is naturally produced cyclic ether and mono terpenoid. Eucalyptal is an ingradient in many brands of mouthwash and cough suppressant. It controls airway mucus hypertension and asthma via anti-inflammatory cytokinene inhibition. Eucalyptol is an effective treatment for nonpurulent rhinosinsnusitis. Eucalyptol reduces inflammation and pain when applied topically. It kills leukemia cells in vitro. [33]

The most common used essential oil extraction method from clove leaves is hydro-distillation (HD) using Clevenger and Soxhlet equipment, however this process requires a long extraction time. Thus, with the development of the current technology, the extraction is based on the renewable technology, namely microwave hydro distillation, ultrasound extraction, subcritical water, and supercritical fluid extraction. But it has some disadvantages such as a lot of solvent volume, high heat temperature, the presence of toxic solvents, and expensive equipment used. While research with microwave extraction, proves that microwave based extraction technology is capable of being applied to different process systems that are more customizable with lower energy consumption and better environmental impingement [34].

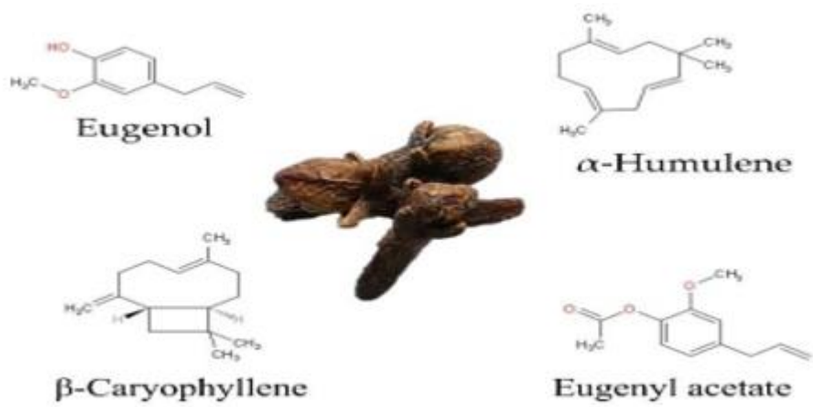

Figure 1. Chemical structure of main compounds of clove essential oil.

1.3. Chemical Structure Clove Essential Oil (CEO):

Syzygium aromaticum L. Belong to the *Myrtaceae* family, which has more than 3000 species and 130–150 genera, such as the myrtle, eucalyptus, clove, and guava families. Clove is an aromatic flower cultivated in Madagascar, Sri Lanka, Indonesia, and China [14,10,6] in India it is cultivated in the hilly areas of Kerala Tamilnadu, and Karnataka. Several reports suggest that *S.aromaticum* L. contains approximately 15–20% wt. of EO. CEO contains a high amount of phenolic compounds with several biological activities, including antibacterial, antifungal, insecticidal, and antioxidant properties [35,14,15,6]. The FDA classifies CEO as generally recognized as safe (GRAS); for this reason, it is used in perfumes, cosmetics, sanitary products, medicines, and foods [15,6].

1.4. Hydro-distillation

Hydro-distillation is also one of the mostly used methods for the extraction of essential oils [10]. During hydro distillation method, powdered sample (100 g dried and ground clove buds) is soaked into water. To carry

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out hydro-distillation, dried clove sample is taken into 500 mL volumetric flask and subjected to hydro-distillation for 4–6 hours. Subsequently, the volatile distillate is collected and saturated with sodium chloride following the addition of petroleum ether or other suitable organic solvent. Later, hydro and ether layers are separated and dehydrated by using anhydrous sodium sulphate. Eventually, the sample is heated in water bath at 60 °C for the recovery of ether and concentration of extract. The average yield of oil using hydro-distillation is about 11.5% whereas reported eugenol concentration is 50.5– 53.5%. However, extraction yield can be increased by reducing the particle size of ground clove buds. [36].

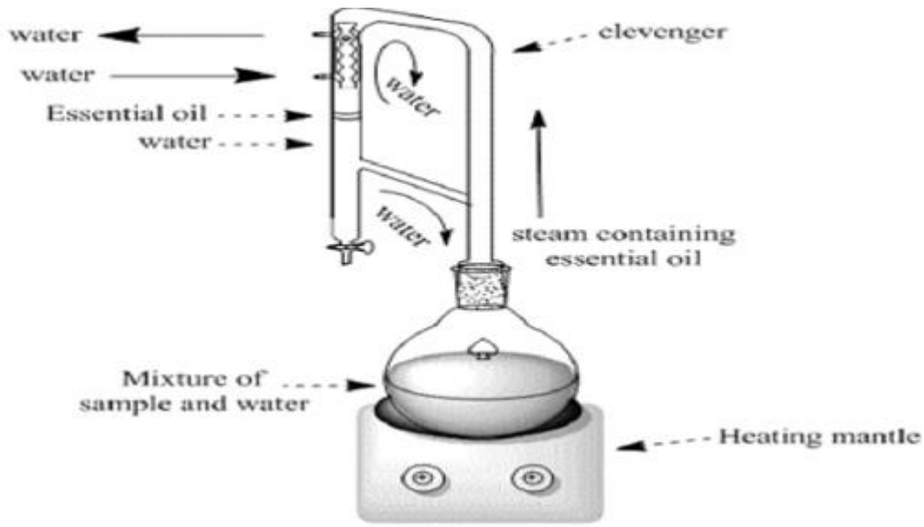

Figure 2. Extraction of clove oil by Hydrodistillation Method

2. MATERIALS AND METHODS:

Clove buds collected from Local Market Generalganj, Kanpur

2.1. Analysis methods of gas chromatography:

Mass spectroscopy GC-MS chromatographic conditions. Analysis was performed with Perkin Elmer clarus 500 (Turbomass software ver. 5.0.0) data handling system with manual injection/injector with mass detector.

Column: Rtx^{R}-5 Capillary column (60m×0.32mm ID X film thickness 0.25 µm) Cross bond® 5% diphenyl siloxane.

Carrier gas:	Helium gas flow rate 1 ml/min
Injector temp (Inlet):	210^0C
Injection volume:	0.2 µl.
Column oven temp:	60^0 for 2min ramp 3^0C/min
Mass range:	40-500 m/z
Solvent delay time:	5 min
Ms scan time:	5.1 min to 55 min.
Ionization mode:	EI^+

Collected sample were crushed properly and weight it. 100 gram sample was used in both methods (Hydro distillation and solvent extraction) n-hexane. Approximately 2 ml oil was extracted per sample. The processes were repeated till sufficient amount of oil extracted. The oil extracted was light in weight and pale in color with sweet smell.

3.1. Result and Discussions:

Samples were analysed by GC and GC-MS techniques, to study of the constituents present in clove essential oil and their medicinal properties. Aim of this study was to analyze the chemical composition of dried clove buds volatile oil by Gas Chromatography-Mass Spectrometry (GC- MS) technique and also show their medicinal properties according to the characteristics of the constituents present in that essential oil. In this experiment the clove buds essential oil was extracted by hydro distillation method.

3.2. Chemical Analysis by GC and GC-MS:

Both the lab extracted sample along with commercial oil sample purchased from General Ganj Kanpur and Kannauj were chemically analyzed using combination of GC and GC-MS.

The results are tabulated in Table-1.

Table: 1 Chemical composition of clove oil by different methods.

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S. No.	Name of Compound	Hydro-distillation	Solvent extraction (n-hexane)	Commercial oil
1.	Limonene	0.198%	0.186%	0.136%
2.	Eucalyptol	3.96%	3.52%	1.6%
3.	Benzyl alcohol	0.65%	0.56%	0.05%
4.	Eugenol	78%	75%	75%
5.	α-caryophyllene	14%	12%	13%
6.	Cis α-bisabolene	1.2%	0.8%	0.4%
7.	3 α-6 methoxy phenyl acetate	0.6%	0.3%	0.17%
8.	Caryophylleneoxide	0.3%	0.21%	0.15%
9.	Furfural	0.06%	0.04%	0.04%
10.	3allyl, 6-methoxyphenol	t	t	t
11.	bicyclo decene	t	t	t
12.	Methyl Benzoate	t	t	t
13.	Tert. Butyl Batene traces.	t	t	t

* t = trace

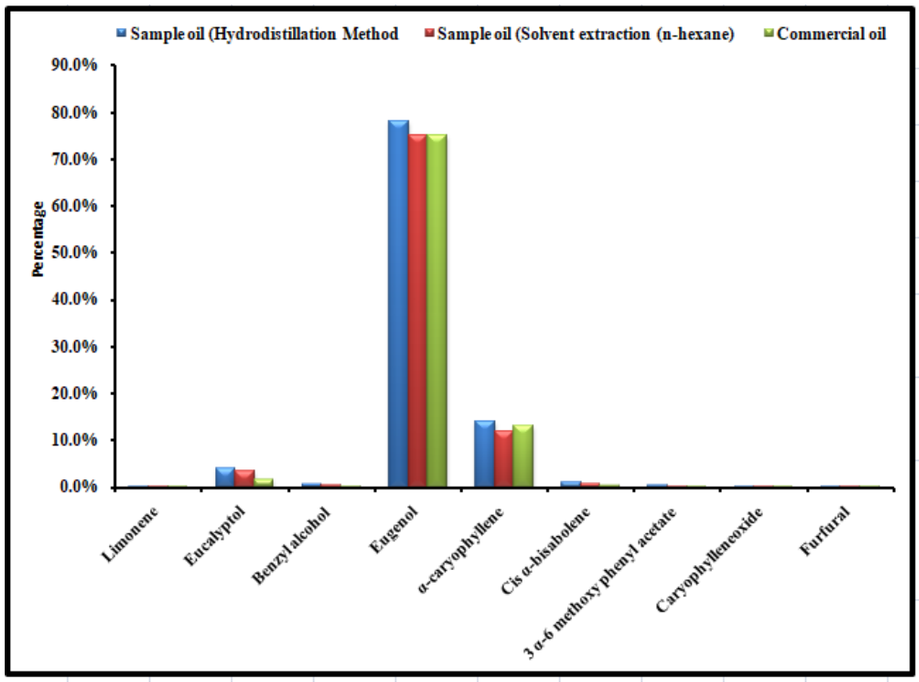

Figure 3. Bar Graph Chemical component of clove oil by different methods.

It was clear from GC/MS analysis report that oil obtained from lab samples had better percentage of Eugenol content than the commercial sample. Eugenol is the main component of clove oil which is responsible for the aroma and medicinal value. So it prooves that lab samples were better than the commercial sample.

3.3. Medicinal value and Antimicrobial activity of CEO:

The antimicrobial activity of CEO was tested against two fungi i.e., *microsporum gypseum* and *Bipolaris specifera*. CEO oil showed positive antifungal activity against these two fungi.

Table 2. Activity of Syzygium aromaticum essential oil against Microsporum gypseum and Bipolaris

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specifera

Clove Oil	Inhibition zone in (mm)		
	4 Days	6 Days	8 Days
microsporum gypseum	55 ± 7.02	57.00 ± 5.18	64.00 ± 3.42
Bipolaris specifera	55 ± 7.02	57.00 ± 5.18	64.00 ± 3.42

Data are multiple of three replicates Value ± SEM
Data include 6mm diameter of sterile disc.

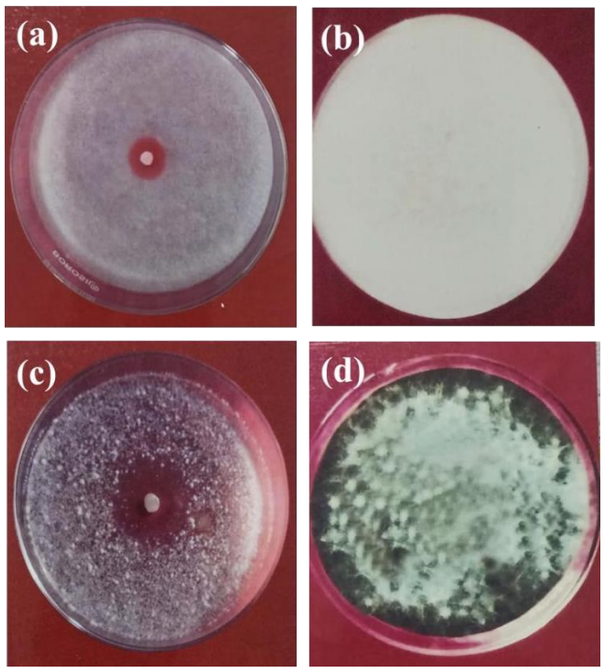

Figure 4. Comparative antimicrobial activity of Syzygium aromaticum essential oil against Microsporum gypseum and Bipolaris specifera (a) Inhibition zone against Microsporum gypseum, (b) Control of Microsporum gypseum, (c) Inhibition zone against Bipolaris specifera, (d) Control of Bipolaris specifera.

4. CONCLUSION:

In this research paper we revealed significant difference between lab sample of clove oil and commercial sample. Hydro distillation method is ecofriendly process without using any solvent as a chemical. It cost is lesser than other two methods. It also restore the quality of the extracted oil. The antimicrobial activity of CEO was tested against two fungi i.e., microsporum gypsum and Bipolarize specifiers. Clove Essential Oil showed positive antifungal activity against these two fungi. It could be concluded that clove represents a very interesting plant with an enormous potential as food preservative and as a rich source of antioxidant, antifungal, antibacterial, analgesic compounds. Its provided biological activities suggest the development of medicinal products for human and animals uses and confirm why this plant has been employed for centuries.

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