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CHEMICAL COMPOSITION, QUALITY CONTROL AND ANTIOXIDANT ACTIVITY OF SOME MARKETED CLOVE OIL SAMPLES AND POSSIBLE ANTI-COVID-19 ACTIVITY OF THE OIL

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ABSTRACT

This work aimed to evaluate the quality of some commercial clove oil products in the Egyptian market either purchased or extracted from clove oil buds. Also, to evaluate the antioxidant potential of these oil samples. Thus quality control of theses oil samples depending on both results. Also, to test the antiviral clove oil activity against SARS-CoV as it was reported as one of the potent antioxidants and its major component; β- caryophellene showed activity against Covid-19. Seven essential oil samples were qualified depending on analyzed using GC/MS analysis by comparing the percentage composition of the main ingredients of clove oil; eugenol, eugenol acetate and β- caryophellene with those reported in pharmacopeia and other references. The antioxidant potential of these samples was performed using DPPH radical scavenging assay using ascorbic acid as a reference standard. The antioxidant potential of these samples had been taken in consideration for quality control of the tested samples. The antiviral activity of clove oil was assessed using Crystal violet assay. The tested samples showed variation either in the number of the peaks in the GC/MS chromatograms and thus was divided into three groups depending on similarity. Also, the percent concentration of each of the eugenol and other main ingredients showed variations. All the tested samples showed antioxidant potential with EC_{50} values ranging from 19.83 to 59.08 µg/ml. Clove oil showed activity against Coronavirus with SI equals 1.95 ng/ml.

KEYWORDS: clove oil, GC/MS, quality control, antioxidant, anticovid-19.

INTRODUCTION

Essential oils are highly appreciated mixtures of secondary metabolites of aromatic plants. Many are used for healthcare in aromatherapy. The oil of clove is extracted from the dried aromatic flower buds of the evergreen trees; *Eugenia caryophyllata* Thunb (=*Syzygium aromaticum* L.) belonging to F. Myrtaceae. The plant is indigenous to the Molucca or Clove Islands. It is widely cultivated in tropical and subtropical countries as in Zanzibar, Pemba, Madagascar, Indonesia, Brazil, Sri-Lanka and Tanzania.[1] Clove oil has received considerable interest due to its wide application in food as flavoring agent, in cosmetics and in oral hygiene products. The FDA classifies it as generally recognized as safe.[2] Due to the increased number of oil suppliers as pharmacies, drug stores, and market, demand for efficient analysis and quality control is increased to ensure good manufacturing practice.

The formulation of medicinal products with adulterated essential oils could be very dangerous and put in serious risk the health of consumers. As an example, clove essential oil is used in dental care pharmaceutical products. Clove oil is usually adulterated either by vegetable oils or by benzyl alcohol.[3] Thus strict controls must be carried out by governmental institution and also by companies working with essential oils as raw materials. The standardization and quality control of essential oils is critical.

According to European Pharmacopoeia, Clove oil is obtained by steam distillation of the dried flower buds of *Syzygium aromaticum* (L.) Merrill et L.M. Perry (*Eugenia caryophyllata* (C. Spreng., Bull. et Harr.). The essential oil of clove is a mixture of different compounds, with the main three active chemical compounds being eugenol, eugenyl acetate and caryophyllene.[4] The major constituent of clove oil is eugenol up to 70-80 %.[5] Clove bud oil, is a colorless or yellow liquid, clove stem oil is pale yellow and it is less expensive than clove oil buds while the clove leaf oil is dark brown in color and it is the main traded clove oil, because it is less expensive than the clove buds. Origin, variety, post-harvest processing, pre-treatment before distillation and distillation method, all are the factors affecting the yield and quality of the produced oil.[6]

Clove is often used as a remedy for diarrhea and for digestive disorders as it is widely used for treatment of dyspepsia and gastritis.[7] It also showed antidiabetic activity.[8] anti-inflammatory and vasodilator activity.[9] The use of clove as analgesic have been reported

since the 13th century, for toothache, join pain and eugenol is the main compound responsible for this activity. Clove oil can also serve as an anesthesia for a variety of fish.[10] It was reported as an inhibitor of platelet aggregation and may act as an antithrombotic agent.[11]

Clove extracts and essential oil showed potent antioxidant activity.[5, 12] A study showed that the antioxidant and antimicrobial activity of clove is higher than many fruits, vegetables and other spices and thus should deserve special attention.[14] Seller et al concluded that clove oil could revert memory and learning deficits caused by scopolamine in short and long term as a result of the reduction in the oxidative stress in mice. Also, its anticancer activity is proven. The cytotoxicity of eugenol is tested and results showed that eugenol presented a notable anti-oxidative potential at all the tested concentrations. It was concluded that the cytotoxic activity of eugenol is stronger than the other tested substances.[15]

Besides, the essential clove oil reported antimicrobial, antifungal, antiviral and insecticidal properties.[16] A study.[17] concluded the antibacterial effect of pure Clove oil or mixed with rosemary oil against *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, E. coli, Proteus vulgaris, Pseudomonas aeruginosa* and the results showed minimum inhibitory concentrations between 0.062% and 0.500% (v/v) which is promising as anti-infection agent or as food preservative. Also, eugenol at 2 µg/mL inhibited the growth of 31 strains of Helicobacter pylori, after 9 h, and thus being more potent that amoxicillin and without developing resistance.[6]

Other uses of clove oil as antipyretic, aphrodisiac, appetizer, expectorant, antiemetic and decongestant were reported.[18]

The clove essential oil is generally recognized as safe substance, the World Health Organization (WHO) established that the daily quantity acceptable of clove per day is of 2.5 mg/kg of weight in humans.[6] The National Toxicology Program based on long term carcinogenicity studies concluded that eugenol is not carcinogenic to rats.[19]

One of the biggest challenges facing scientists from all over the world today is to find and develop effective drugs to fight SARS-CoV-2. Parallel to intensive efforts that has been done to find a vaccine or a cure for the disease, alternative treatments are also being tested. FDA-approved drugs with different therapeutic uses are being re-examined for their possible

activity against SARS-CoV-2. Among those are natural products especially essential oils; which have a wide range of biological effects, including antiviral activity. A previous study[20] concluded the antiviral efficacy of essential oil components has been determined specifically against SARS-CoV-2. Several compounds found in volatile oils, including thymol, have been studied. For this purpose, the recently determined crystal structure of the SARS-CoV-2 spike glycoprotein (S) was used. Based on the binding affinities of the compounds against the receptor binding domain of the S1 glycoprotein, cuminal, carvacrol, myrtanol, and pinocarveol were found to be highly active.[21] Clove oil is known as a powerful antioxidant and has antiviral activity but still not tested against SARA-CoV virus.

The present study aimed to evaluate the quality of 7 clove oil samples from different suppliers either by extraction from its flower buds or oil samples already extracted and marketed in Egypt by determination of their chemical composition. Also to determine, compare oils antioxidant potential and correlate them with oil composition. Both oil composition and antioxidant activity were used to compare and evaluate samples quality. Also to evaluate the effectiveness of clove oil against SARS-COVID virus.

MATERIAL AND METHODS

General Experimental Procedures

TLC of the purchased essential oil samples together with that was extracted was done on precoated silica gel 60 F254 plates (Germany).

Camag, Switzerland UV Lamp was used for UV detection of the TLC plates.

GC/MS analysis was performed on Perkin Elmer model: Clarus 580/560 S).

Clevenger apparatus was used for essential oil extraction.

Spectrophotometer (Optima SP-300, Japan).

Chemicals and solvents were purchased from Sigma Chemical Co. (St., Louis, USA.

Eugenol was obtained from Sigma-Aldrich.

DPPH was purchased from Sigma Chemical Co. (St. Louis, USA).

Plant Material

The flowering buds of clove (Eugenia caryophyllus = Syzygium aromaticum) belonging to the Family Myrtaceae were purchased from a local market. Botanical identification has been confirmed by macroscopic and microscopic examinations.

Extraction of the Essential Oil

Clove flower buds (100 gm) were purchased from local market in Egypt and crushed using mortar and pestle just prior to extraction was subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The obtained essential oil was dried over anhydrous sodium sulfate and stored in amber vials with screw cap in a refrigerator at 4 °C prior to analysis. The extracted oil was labeled as Supplier 1 and stored in amber vials for use.

Commercial oil Samples

Six commercial clove oil samples were purchased from the Egyptian market. The oil samples were coded Supplier 2, Supplier 3, Supplier 4, Supplier 5, Supplier 6 and Supplier 7. The sample coded Supplier 4 was prepared by cold press extraction method.

TLC of Clove Oil

All oil samples were spotted on gel F percolated plates and developed using in n-hexane: acetone (9:1) and n-hexane: ethylacetate (4:1) as mobile phase. Plates were visualized under UV and sprayed with ferric chloride.

Clove Oil Samples were Co-Chromatographed with authentic Eugenol.

All essential oil samples (Supplier 1, 2, 3, 4, 5, 6 and 7) showed eugenol recognized by the same authentic R_f = 0.35. Eugenol spot was visualized under UV but appear as green black spot after spraying with ferric chloride reagent. The intensity of eugenol spot varied as being more intense in samples 1 and 6 and 7. Essential oil samples showed 4 spots in all samples except Supplier 3 that shows only 3 spots. R_f values in n-hexane: acetone (9:1) 0.65, 0.4, 0.35 and 0.18. The four spots appeared after spraying with $Fecl_3$ as brown, blue and greenish black and green respectively in n-hexane: acetone (9:1).

Gas Chromatography/Mass Spectroscopy of the Clove Oil Samples

The chemical composition of the tested samples were performed using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C /min to250 °C hold for 2 min. increased to the final temperature 300°C by 30°C /min and hold for 2 min. The temperatures of the injector and MS transfer line were kept at 270, 260°C respectively. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 µl were

injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparing their mass spectra with those of WILEY 09 and NIST 14 mass spectral database.

Antioxidant activity (DPPH radical‑scavenging assay)

The DPPH radical scavenging assay was determined for all the seven samples according to the method described previously by Tien et al.[22]

Assessment of the antioxidant activity of both the isolated and commercial clove oil samples was done by the radical scavenging effect of the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical using ascorbic acid as a standard. The antioxidant radical scavenging effects on DPPH are due to their hydrogen donating ability which causes an absorbance drop at 517 nm. Serial dilutions (25-100 µg/ml) of the tested oil samples were measured by the same assay to obtain EC50 (effective concentration at which the DPPH scavenging activity being half its maximal activity).

EC50% values (µg /ml) means the effective concentration at which the DPPH scavenging effect being 50%. It was obtained by interpolating from the linear regression analysis.

Antiviral / Human Coronavirus 229E (Crystal Violet Assay)

The CPE-inhibition assay (the first assay developed to evaluate whether a substance showed antiviral activity) will be used to identify potential antivirals against human coronavirus 229E. The dose-response assay was designed to determine the range of efficacy for the chosen antiviral, i.e. the 50% inhibitory concentration (IC50), as well as the range of cytotoxicity (CC50). This assay is critical for determining antiviral efficacy in cell culture systems. Nawah-Scientific, Egypt, provided the 229E virus and Vero E6 cells. Vero E6 cells were grown in DMEM medium supplemented with 10% fetal bovine serum and 0.1% antibiotic/antimycotic solution. The antibiotic & antimycotic solution, trypsine-EDTA, fetal bovine serum, and DMEM medium were provided Gibco BRL (Grand Island, NY, USA). The crystal violet method was used to evaluate antiviral activity and cytotoxicity assays according to Schmidtke et al.[23] In brief, vero E6 cells were seeded into a 96-well culture plate at a density of 2x104cells/well one day before infection. The culture medium was removed after 24 hrs and the cells were washed with phosphate-buffered saline. The infectivity of coronavirus 229E was determined using the crystal violet method, which

monitored CPE and allowed the percentage of cell viability to be calculated. 0.1 mL of diluted virus suspension of 229E virus containing CCID50 (50 percent cell culture infective dose of virus stock was added to mammalian cells. This dose was selected to produce the desired CPEs. For compound treatments, 0.01 mL of medium containing the desired compound concentration was added to the cells. Each test sample's antiviral activity was determined using a two-fold diluted concentration range of 0.00001-1 µg/mL. The virus controls (virus-infected, nondrug-treated cells) and cell controls (non-infected, nondrug treated cells). For 72 hrs, culture plates were incubated at 37oC in 5% CO2. The development of cytopathic effect was monitored by light microscopy. Following a PBS wash, the cell monolayers were fixed and stained with a 0.03% crystal violet solution in 2% ethanol and 10% formalin.

After washing and drying the optical density of individual wells was quantified spectrophotometrically at 540/630 nm. The percentage of antiviral activities of the tested oil was calculated according to Pauwels et al.[24] using the following equation: antiviral activity= [(mean optical density of cell controls−mean optical density of virus controls)/ (optical density of test−mean optical density of virus controls)] ×100% using the DIAS. Based on these results, the 50% CPE inhibitory dose (ID50) was calculated.

Before this assay, the cytotoxicity of the oil is to be tested. Thus cells were seeded at a density of $2x10^4$ cells/well in a 96-well culture plate. After 24 hrs, the culture medium containing serially diluted samples was added to the cells and incubated for 72 hours before being removed and the cells washed with PBS. The steps were carried out in the same manner as described above for the antiviral activity assay.

Calculations are made by determining SI (selectivity index). It was determined by the ratio of CC50 (concentration of 50% cellular cytotoxicity) to IC50 (50% inhibition concentration)

To give antiviral activity, this value must be higher than one. The ideal situation is to have IC50 below the CC50 value. This means that the drug kill the virus before killing the host.

Statistical Analysis

Antioxidant assays were conducted in triplicate. Data were expressed as mean ± standard deviation (SD). Data were analyzed using Graph-pad PRISM Software.

RESULTS

The yield of the clove buds isolated essential oil was about 5.76%.

The oil samples are heavier than water, dark yellow in color. The purchase oil sample coded supplier 3 is lighter in density and lighter in color. All have characteristic clove odor except sample coded supplier 3 which has very faint odor.

Chemical Composition of Clove Oil Samples

The tested seven samples showed different numbers of peaks in the GC/MS diagram. Samples can be grouped into three different groups according to their GC/MS diagrams. Samples coded Supplier 1 & Supplier 6 and supplier 7 showed similar behaviors in GC/MS diagrams as they showed only five peaks. Thus to be represented by Supplier 1diagram in Fig 1. Also, samples coded Supplier 2 & Supplier 4 and supplier 5 showed similar behaviors in GC/MS diagrams despite of disappearance of levomethanol peak (at R_t 8,25) in GC/MS diagram of sample coded supplier 2. Thus to be represented by Supplier 4 diagram in Fig 1. On the other hand, sample coded supplier 3 showed somewhat different pattern in GC/MS diagram, thus to be represented in Fig 1.

GC/MS diagrams representing the three similar groups represented by supplier 1 & supplier 3 and supplier 4 are shown in Fig. 1.

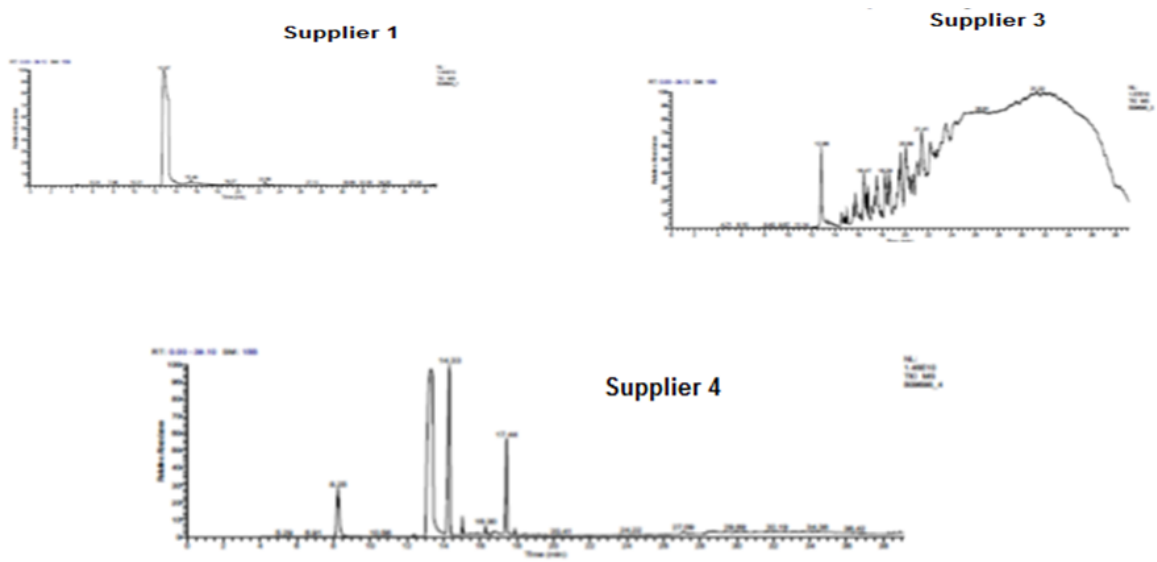
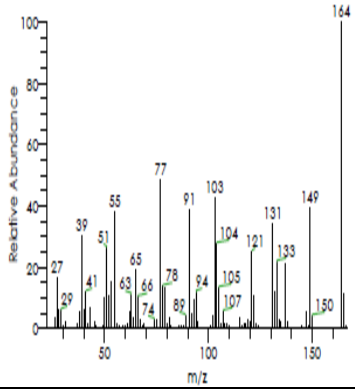
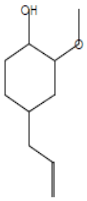
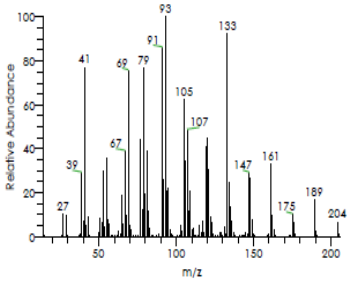
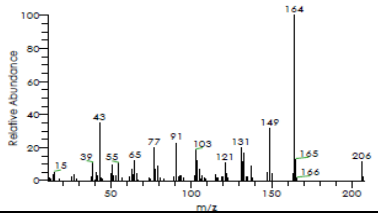
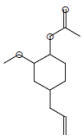
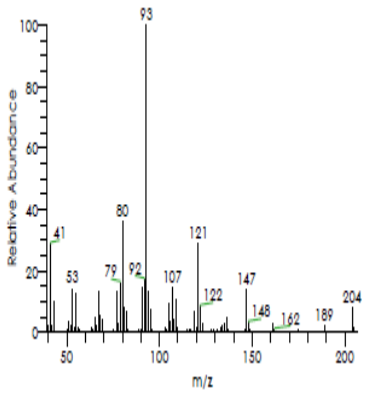
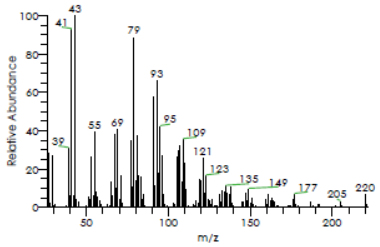
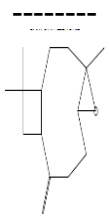
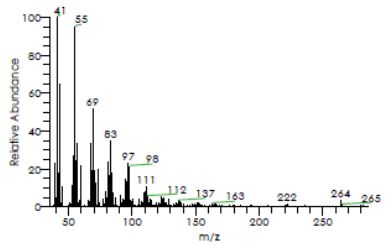
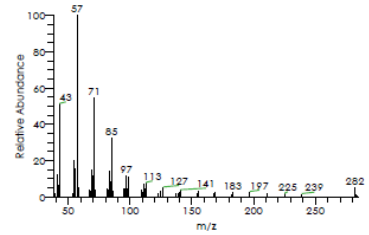
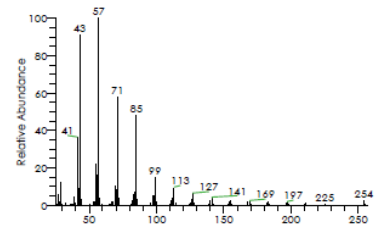
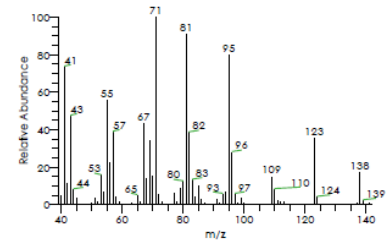
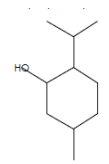


Fig. 1: GC/MS diagrams of clove oil samples code supplier 1 &supplier 3 and supplier 4 analyzed for quality control and represented the three differented resulted groups in gc/ms diagrams.

Clove oil is a mixture of different compounds, with the three main active ingredients being eugenol, eugenyl acetate and β-caryophyllene. The major compounds in clove oil samples either extracted or purchased are represented in Table 1.

Table 1: Percentage of Major Compounds In GC/MS of The Seven Tested Clove Oil Samples Extracted and Purchased.

Compound /	% composition						
Mass spectrum	Supplier 1	Supplier 2	Supplier 3	Supplier 4	Supplier5	Supplier 6	Supplier 7
Eugenol 	**79.27** 	**62.66**	**8.02**	**60.43**	**61.1**	**72.4**	**77.22**
β-Caryophylline 	**18.16** 	**18.11**	**----**	**20.28**	**18.3**	**13.3**	**15.2**
Eugenol acetate 	**0.59** 	**0.33**	**0.35**	**0.37**	**0.31**	**0.41**	**0.44**
α-Humulene 	**0.59**	**1.65**	**------**	**1.55**	**1.60**	**0.62**	**0.76**

Caryophylline oxide 		**8.35**	**-----**	**9.47**	**8.66**	**----**	**------**
9-Octadecanoic acid 	**1.05**	**0.21**	**0.85**	**0.26**	**0.22**	**-----**	**------**
Eicosane 	**------**	**1.23**	**1.36**	**1.36**	**1.26**	**1.11**	**0.95**
9-Octadecane 	**------**	**1.31**	**4.54**	**1.55**	**1.32**	**------**	**-----**
Levomenthol 	**-----** 	**-----**	**-----**	**5.93**	**4.20**	**----**	**------**

Quality control of the oil samples will be assessed depending on the percent concentration of the principle compound eugenol and to lower extend other compounds such as β-Caryophylline and eugenol acetate compared with those described in the European Pharmacopeia and literatures.

Table 2: Decrease of DPPH absorbance (%) and EC_{50} values for the seven tested clove oil samples.

Supplier	The decrease of DPPH absorbance % mean ± SD (*n* = 3)	EC_{50} (µg/ml) mean ± SD (n = 3)
1	77.1 ± 1.34	20.78± 0.2
2	54.33 ± 0.76	55.8± 0.8
3	38.63 ± 0.08	59.08± 0.77
4	74.13 ± 0.37	30.9± 0.69
5	59.76 ± 0.89	34.1± 0.88
6	72.11 ± 0.33	19.83± 0.07
7	69.89± 0.21	19.89± 0.33
Ascorbic acid (standard)	81.60 ± 0.82	8.59± 0.34

The Antioxidant Potential of the Tested Clove Oil Samples

All oil samples showed antioxidant activity varied to different extents. Results are tabulated in Table 2. The percentage of scavenging ability of all the tested seven clove oil samples using DPPH. Sample coded supplier 1 showed the highest antioxidant potential (77.1± 1.34) whileas sample coded supplier 3 showed the lowest ones (38.63 ± 0.08).

Results of Antiviral / Human Coronavirus 229E

The results of the 50% cytotoxic concentrations (CC50) and the 50% inhibitory concentration (IC50) were determined using GraphPad PRISM Software (Graph-Pad Software, San Diego, USA).

SI= CC50 /IC50= 215.4/135.8 = 1.59 ng/ml. This means that the clove essential oil is effective against Coronavirus.

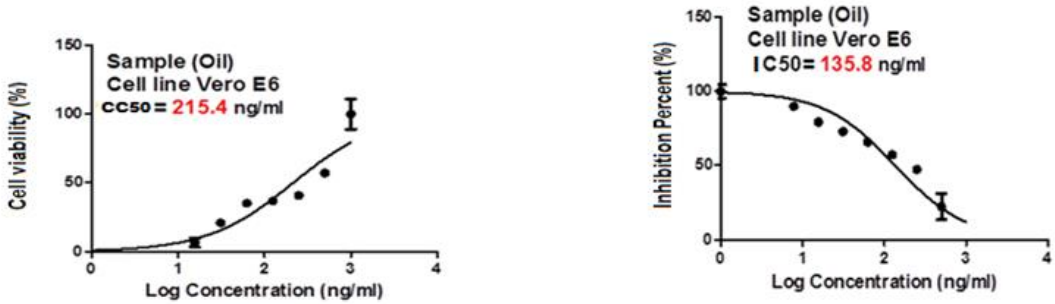


Fig. 2: Graphs of cytotoxicity Concentration 50% (CC50) and the 50% inhibitory concentration (IC50) on Vero E6 cells and 229E.

DISCUSSION AND CONCLUSION

The oil yielded from extraction of the dried clove buds was in agree with yields acquired by several studies that was varying from 0.18 to 7.6%.[24–26] It is known that clove oil is heavier than water as its density is 1.04 g/mL at 25^{o}c. Sample coded supplier 3 was shown to be admixed with other oils and extracted from exhausted clovebuds. This was supported by its physical character as lighter density, lighter color and very faint clove odor. This was supported by chemical composition analysis by GC/MS as eugenol percent composition was very low (8.20), absence of β-caryophyllene, α-humulene and β-caryophyllene oxide. Also, very low concentration of eugenol acetate (0.35%).

The difference in the number of identified compounds in GC/MS may refer to the age of the clove buds from which the essential oil was extracted. A previous work[27] showed that the flowering buds of young trees produced higher yield than that of the mature ones. Alfikri et al (2020) showed that, in GC-MS, the main bioactive compound of clove oil; eugenol is highest at the flowering stage in young than mature trees. This may explain the percent variation of eugenol in the tested samples as oil was extracted from different growth variation stages of the buds.

According to European pharmacopea, the main characteristic constituents of the essential oil are phenylpropanoids eugenol (75-88%), β-caryophyllene (5-14%) and acetyleugenol (4–15% of the oil); minor constituents, less than 1%, are 2-heptanone, ethyl hexanoate, humulenol; sesquiterpenes, α-humulene, α-humulene epoxide, β-humulene, β-caryophyllene oxide and α-cubebene.

The major constituent of clove oil; eugenol up to 70-80 % [28- 30]. Some samples as coded supplier 2, 4 and 5 showed slight decrease in the percent of eugenol (62.66, 60.43 and 61.1% respectively). Sample coded Supplier 3 showed dramatic decrease in eugenol content (8.20%). This supports adulteration of this clove oil sample and thus must be rejected.

Eugenol acetate is known to exhibit antibacterial, antioxidant, and anti-virulence activities.[31]

The terpene β-caryophyllene due to its unique ability to bind with CB2 receptors, it has potent anti-inflammatory, antimicrobial, antibacterial, and antioxidant properties. Thus, known to help relieve anxiety and pain**.** The combination of Humulene with β-caryophyllene have anti-inflammatory properties that make it practical for treating arthritis, bursitis, and

fibromyalgia.[32] β-caryophyllene in all the tested samples (except sample coded supplier 3) was found to be in agree with the standard pharmacopeal limits.

Indeed, sample coded supplier 3 must be rejected and withdrawn from the market as it showed very low concentration of eugenol (8.2%). Also, it showed absence of the peaks of β-caryophyllene, α-humulene and caryophellene oxide.

Levomenthol is a levo isomer of menthol, an organic compound made synthetically or obtained from peppermint oil. As other components of menthe essential oil is not found in Gc/Ms chromatogram, synthetic levomenthol is add to sample coded supplier 4. Levomenthol/clove oil combination is used for occasional minor irritation, pain and headache. Also this combination was shown to have anaesthetic effect.[33]

Cold pressed clove oil was reported to posses stronger radical scavenging and antioxidant activity than oil extracted by hydrodistillation. This may explain the higher antioxidant activity of sample coded supplier 4.[34]

For conclusion, we can say that all the tested samples complied with the monograph of the European Pharmacopoeia [Clove oil] except sample coded Supplier 3.

Also, the EC50 values of all the tested oil samples are accepted as showed potent antioxidant activities. Previous results for *S. aromaticum* essential oil was 3.47 µg/mL in comparison to 13.98 µg/mL for ascorbic acid.[14] Alfikri, F et al[27] showed that DPPH scavenging activity of clove bub oil had IC_{50} range 15.80-108.85 μg/mL, with the highest antioxidant activity at the flowering stage of young trees than mature buds.

In a previous work, clove oil was evaluated by employing various in vitro antioxidant assay and shown to be very effective.[18] United States Food and Drug Administration recognized clove oil as safe substance when administered at levels not exceed 2.5 mg/kg body weight for humans. Such consumption levels of clove oil can reduce many health risks due to its potent antioxidant activity.

A previous work[34] speculate that β-caryophyllene; a major component in clove oil has potential to be investigated against COVID-19 and will inspire further preclinical and clinical studies. Other previous studies concluded that clove oil is one of the most antioxidant volatile oils.[16] This work concluded that clove oil is effective against SARS-CoV virus as its

selectivity index that measures the window between cytotoxicity and antiviral activity equals 1.95 ng/ml.

RECOMMENDATION

Quality control of the marketed essential oils is a must as they may be used either internally or externally and thus affects human health if adulterated by harmful substances or even if subqualified. GC/MS is an effective method for determining the quality control of essential oils despite being expensive.

Due to its potent antioxidant potential, clove oil can be added to prolong the shelf life of foods and pharmaceuticals.

This work concluded that clove oil is effective against Coronavirus. Such in-vitro studies are promising but need further studies.

CONFLICT OF INTEREST

None.

FINANCIAL SUPPORT

None.

REFERENCES

1. Evans WC. Trease and Evans Pharmacognosy. 15th Edition. WB. Saunders, 2008; 250-274.
2. Haro-González JN, Castillo-Herrera GA, Martínez-Velázquez M and Espinosa-Andrews H. Clove Essential Oil (Syzygium aromaticum L. Myrtaceae): Extraction, Chemical Composition, Food Applications, and Essential Bioactivity for Human Health. Molecules, 2021; 26(21): 6387.
3. Jentzsch P, Gualpa F, Ramos LA, Ciobotă V. Adulteration of clove essential oil: Detection using a handheld Raman spectrometer. Flavour Fragrance J., 2017; 33(2): 184-190.
4. Jirovetz L, Buchbauer G, Stoilova I et al. Chemical composition and antioxidant properties of clove leaf essential oil. J Agric Food Chem, 2006; 23-54(17): 6303-6307.
5. Nurdjannah N, Bermawie N. Clove In: Peter K.V. Handbook of herbs and spices. Woodhead Publishing Series in Food Science, Technology and Nutrition Cambridge: Woodhead Publishing Limited, 2001; 281-304.

6. Singh J, Baghotia A, Goel SP. Eugenia caryophyllata Thunberg (Family Myrtaceae): a review. Int. J. Res. Pharm. Biomed. Sci, 2012; 3(4): 1469–75.
7. Prasad, RC, Herzog, B and Boone, B et al. An extract of Syzygium aromaticum represses genes encoding hepatic gluconeogenic enzymes. J. Ethnopharmacol, 2005; 96: 295–301.
8. Yun s, lee m, lee K et al. Quantitative Analysis of Eugenol in Clove Extract by a Validated HPLC Method. Journal of AOAC international, 2010; 93(6): 1806-10.
9. Hekimoğlu MA, Ergun M. Evaluation of clove oil as anaesthetic agent in fresh water angelfish, Pterophyllum scalare. Pak J Zool, 2012; 44(5): 1297–1300.
10. Mbaveng AT and Kuete V. in Medicinal Spices and Vegetables from Africa, 2017.
11. Kamatou GP, Vermaak I and Viljoen AM. Eugenol from the remote Maluku islands to the international market place: a review of a remarkable and versatile molecule. Molecules, 2012; 17: 6953-81.
12. Cortés-Rojas DF, Fernandes de Souza CR, and Oliveira WP. Clove (Syzygium aromaticum): a precious spice. Asian Pac J Trop Biomed, 2014; 4(2): 90–6.
13. Halder S, Mehta AK, Kar R et al. Clove oil reverses learning and memory deficits in scopolamine-treated mice. Planta Med, 2011; 77(8): 830–34.
14. Selles SA, Kouidri M, Belhamiti BT et al. Chemical composition, in-vitro antibacterial and antioxidant activities of Syzygium aromaticum essential oil Journal of Food Measurement and Characterization, 2020; 14: 2352–58.
15. Chaieb K, Zmantar T, Ksouri R et al. Antioxidant properties of the essential oil of Eugenia caryophyllata and its antifungal activity against a large number of clinical Candida species. Mycoses, 2007; 50: 403–6.
16. Park MJ, Gwak KS et al. Antifungal activities of the essential oils in Syzygium aromaticum (L.) Merr. Et Perry and Leptospermum petersonii Bailey and their constituents against various dermatophytes. J Microbiol, 2007; 45(5): 460–65.
17. Ali SM, Khan AA, Ahmed I et al. Antimicrobial activities of eugenol and cinnamaldehyde against the human gastric pathogen Helicobacter pylori. Ann Clin Microbiol Antimicrob, 2005; 4: 20.
18. Gülçin I, Elmastaş M, Aboul-Enein HY. Antioxidant activity of clove oil-A powerful antioxidant source. Arab J Chem, 2012; 5(4): 489–99.
19. Ghosh R, Nadiminty N, Fitzpatrick JEet al. Eugenol causes melanoma growth suppression through inhibition of E2F1 transcriptional activity. J Biol Chem, 2005; 280(7): 5812–19.

20. Yadalam PK, Varatharajan K, Chopra P. Antiviral Essential Oil Components Against SARS-CoV-2 in Pre-procedural Mouth Rinses for Dental Settings During COVID-19: A Computational Study. Front. Chem., March, 2021.
21. Alitonou AG, Tchobo FP, Avlessi F et al. Chemical and biological investigations of Syzygium aromaticum L. essential oil from Benin. Int. J. Biol. Chem. Sci, 2012; 6(3): 1360–67.
22. Tien YY, Ng CC, Chang CC, et al. Studies on the lactic- fermentation of sugar apple (Annona squamosa L.) puree. J. Food. Drug. Anal, 2005; 13(4): 377–81.
23. Schmidtke M, Schnittler B, Jahn A, Stelzner et al. A rapid assay for evaluation of antiviral activity against coxsackie virus B3, influenza virus A, and herpes simplex virus type 1. Journal of Virological Methods, 2001; 95(1-2): 133-43.
24. Pauwels R, Balzarini J, Baba M et al. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J Virol Methods, 1988; 20(4): 309-21.
25. Rodríguez O, Sánchez R, Verde M et al. Obtaining the essential oil of Syzygium aromaticum, identification of eugenol and its effect on Streptococcus mutans. J. Oral Res, 2014; 3(4): 218–24.
26. Saeed A, Shahwar D, Evaluation of biological activities of the essential oil and major component of Syzygium aromaticum. J. Anim. Plant Sci, 2015; 25(4): 1095–99.
27. Alfikri FN, Pujiarti R, Wibisono MG, Hardiyanto EB. Yield, Quality, and Antioxidant Activity of Clove (Syzygium aromaticum L.) Bud Oil at the Different Phenological Stages in Young and Mature Trees. Scientifica Jun 2, 2020; 9701701.
28. Santin JR and Schmiedebergs N. Gastroprotective activity of essential oil of the Syzygium aromaticum and its major component eugenol in different animal models. Arch. Pharmacol, 2011; 383: 149–58.
29. Rani B, Kachhawa G. R., Yadav R. K., et al. GC-MS analysis of clove (Syzygium aromaticum) bud essential oil from Java and Manado. Int. J. Res. Pharmacol. Pharmacother, 2012; 1: 230–33.
30. Prianto H, Retnowati R, Juswono UP. Isolasi dan karakterisasi dari minyak bunga cengkeh (Syzigium aromaticum) kering hasil distilasi uap. Jurnal Ilmu Kimia Universitas Brawijaya, 2013; 1: 269–75.
31. Musthafa KS, Voravuthikunchai S. Anti-virulence potential of eugenyl acetate against pathogenic bacteria of medical importance. Antonie van Leeuwenhoek, 2015; 107(3).

32. Jha NK, Sharma C, Hashiesh HM, Arunachalam S et al. β-Caryophyllene, A Natural Dietary CB2 Receptor Selective Cannabinoid can be a Candidate to Target the Trinity of Infection, Immunity, and Inflammation in COVID-19. Front Pharmacol, 2021; 12; 590201.
33. Hoshiba MA, Dias RMS, Moreira KMF, Tamajusuku A. Clove oil and menthol as anesthetic for platy. Nota de Pesquisa, 2015.
34. Devkota HB, Devkota A. Cold pressed clove (Syzygium aromaticum) oil. Cold Pressed Oils: Bioactive Natural Products. project: Chemistry and Pharmacology. Lab: Bioactive Natural Products Laboratory August, 2020.