Characterization of Essential Oil Composition of *Syzygium aromaticum Linn.* (Clove) by GC-MS and Evaluation of its Antioxidant Activity

Javed Ahamad[1]

Abstract

Background: *Syzygium aromaticum* Linn. (Clove, Family: Myrtaceae) is traditionally used as a spice and condiment; and medicinally used as a dental analgesic, carminative, and antiseptic. The Clove buds are widely used as a spice in Kurdish foods and are abundantly available in the local market; the quality of Clove available in the local market of Erbil has not been studied till now. Therefore, the aim of current research is to characterize essential oil composition by gas-chromatography mass spectroscopy (GC-MS) and evaluate its antioxidant potential by the DPPH method. **Results:** The GC-MS analysis of Clove essential oil resulted identification of 37 chemical compounds which constitute about 99.49% of total essential oil. Clove essential oil was found rich in eugenol (59.87%), caryophyllene (23.58%), α-selinene (4.67%), α-terpinyl acetate (4.12%), and humulene (3.74%). The Clove essential oil was found potent antioxidant with a maximum inhibition of 90.94% and it was found comparable with standard antioxidant compounds such as ascorbic acid (92.94%), and gallic acid (87.80%) inhibition. **Conclusion:** The present study explores the essential oil composition of Clove found in the Kurdistan region and results also show its essential oil has potent antioxidant activity.

Keywords: *Syzygium aromaticum*, Clove, Myrtaceae, Kurdistan, GC-MS, DPPH, Antioxidant.

Significance | The study of Clove essential oil as an antioxidant drug.

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1. Introduction

Syzygium aromaticum Linn. (Clove; Family: Myrtaceae) is traditionally used as a dental analgesic, carminative, and natural antiseptic (Chaieb et al., 2007; Cortés-Rojas et al., 2014). Clove is a well-known spice, and widely used in Kurdistan and locally it is known as *Kerenfil*. The pharmacological activities of Clove are due to the presence of large quantities of essential oil which is rich in eugenol, caryophyllene, and eugenol acetate (Haro-González, et al., 2021; Rosarior et al., 2021). Clove buds also contain other many phytochemicals such as saponins (Batiha et al., 2020), alkaloids (Hemalatha et al., 2016), flavonoids (Manivannan et al., 2022), steroid and glycoside (Cortés-Rojas et al., 2014), and tannins (Mostafa et al., 2023) responsible for its pharmacological actions. Clove essential oils have been reported to have several bioactivities such as antioxidant (Batiha et al., 2020; Rosarior et al., 2021), antimicrobial (Somrani et al., 2022; Batiha et al., 2020), anticancer (Han and Parker, 2017), analgesic (Selka et al., 2021), antispasmodic (Batiha et al., 2020), antiseptic (Oliveira et al., 2020; Batiha et al., 2020), antifungal (Biernasiuk et al., 2022; Kalemba, & Kunicka, 2003), antiviral and anti-SARS-CoV-2 (Manivannan et al., 2022; Cortés-Rojas et al., 2014), and anti-inflammatory (Han, & Parker, 2017; Rosarior et al., 2021).

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To check the quality of herbal drugs containing essential oil, gas-chromatography mass spectroscopy (GC-MS) has emerged as an important tool. In this method GC separates the chemical compounds based on their mass and Mass spectroscopy identifies them based on their mass/charge ratio (*m/z*) (Ahamad et al., 2020; Ahamad, & Uthirapathy, 2021). GC-MS techniques nowadays are applied successfully for the identification and quantification of low molecular weight natural compounds such as essential oils, fixed oils, amino acids, proteins, etc. (Van Asten, 2002; Zhang et al., 2021). *S. aromaticum* is widely used as a spice in Kurdish foods and is abundantly available in the local market, the quality of Clove available in Erbil (Kurdistan Region of Iraq) has not been studied till now. Therefore, the aim of the present study is to characterize essential oil composition by GC-MS and evaluate its antioxidant potential by the DPPH method.

2. Material and Methods

2.1. Plant Material and Chemicals

The dried buds of *Syzygium aromaticum* (500 g) were collected from the local market in Erbil, Kurdistan Region, Iraq. The plant sample was identified and authenticated by a Taxonomist and a voucher specimen was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Tishk International University, Erbil, Iraq. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), ascorbic acid, gallic acid, methanol, and ethanol were procured from Danar Company For General Trading and Contracting Ltd, Iraq.

2.2. Isolation of Essential Oil from Clove Buds

For the isolation of the essential oil from Clove buds, the hydrodistillation method was applied. The Clove buds (250 g) were kept in RBF of the Clevenger apparatus with distilled water (500 ml) and 2 ml of glycerin. The experiment was run for 6 hours, after that we collected essential oils and kept them in a refrigerator at 4 °C until further use.

2.3. GC-MS Analysis and Identification of Chemical Constituents

The Clove essential oil composition was determined by the Agilent Bench Top GC-MS (Agilent Technologies, Wilmington, DE, USA). The GC-MS with a capillary column of DB-5 glass (30 m × 0.25 mm i.d.; film thickness of 0.25 μm) and helium was used as carrier gas at a flow rate of 1 ml/min. The temperature of the GC-MS oven was set at 50 °C for 1 min and then isothermally kept for 2 min at 320 °C, while the injector port was maintained at 280 °C. The clove essential oil was injected (0.1 μL) after mixing with hexane (1:1) and the split ratio was kept at 1:5. Data capture took place at 70 eV using scanning times of 1.5 sec in the mass range of 50-1000 amu and run time was kept upto 37 min. The chromatography and mass spectra were handled with Chem station software (Agilent Technologies, Wilmington, DE, USA).

The individual peaks/constituents were identified by comparison of their Kovats Index (K.I.) with those of the literature. Further identification of chemical constituents was made by comparison of the fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NIST, NBS 54 K.L, WILEY8 libraries, and published literature (Adam, 2007; Ali, 2001; Zhang et al., 2021; Hatami et al., 2019; Kennouche et al., 2015; and González-Rivera et al., 2016). The percent composition of each compound was calculated based on the area of respective peaks.

2.4. Assessment of Antioxidant activity by DPPH method

The antioxidant activity was performed as per the method described by Hamad et al., (2013). Clove essential oils ranging in concentration from 1000 to 62.5 μl/ml were assessed for antioxidant activity by using the DPPH method. Diluted solutions of plant extracts (1 ml each) were mixed with 1 ml of methanolic solution of DPPH (1 ml/ml concentration). After 30 min incubation in darkness at room temperature, the absorbance was recorded at 517 nm. The control sample contained all the reagents except the plant extracts. The percentage inhibition was calculated using the following equation.

$$\% \, Inhibition = \frac{A\ control - A\ test}{A\ control} \times 100$$

3. Results

3.1. GC-MS Analysis Essential oil of S. aromaticum

The essential oil of Clove bud was isolated by hydrodistillation method using Clevenger apparatus. The yield of Clove essential oil was found to be about 15.67%, and the isolated essential oil was colorless with a strong aromatic odor. The chemical composition of the essential oil of Clove bud was analyzed by GC-MS method and results were presented in Table 1 and Figure 1. A total of 37 chemical compounds were identified in the essential oil of Clove essential oil which represents about 99.49% of the total essential oil. Eugenol (59.87%), *β*-Caryophyllene (23.58%), α-selinene (4.67%), α-terpinyl acetate (4.12%), and humulene (3.74%) were found as major chemical constituents in the essential oil of Clove (Figure 2). The minor compounds of Clove essential oil were found as α-terpineol (0.91%), *D*-limonene (0.40%), *p*-cymene (0.26%), γ-muurolene (0.21%), γ-terpinene (0.13%), geranyl acetate (0.12%), 2-nonanone (0.11%), linalool (0.11%), terpinolene (0.10%), and germacrene B (0.10%).

The present study results on chemical composition of essential oils of Clove is compared with previously published research. In Table 2, the composition of the essential oil of Clove is compared with the previous studies done by Hatami et al., (2019); Kennouche et al., (2015); and González-Rivera et al., (2016). The major chemical compounds such as eugenol and caryophyllene content of the current study are found comparable with the previous studies. In the present study, α-selinene (4.67%), and α-terpinyl acetate (4.12%) were found as major constituents that are absent in the

Table 1. Chemical constituents of essential oil of *S. aromaticum* (Clove)

S. No.	Compounds	R. Time	K. Index	Content (%)
1.	2-Heptanone	5.752	891	0.06
2.	*α*-Pinene	7.105	933	0.04
3.	Sabinene	8.553	968	0.03
4.	Myrcene	9.246	983	0.06
5.	α-Phellandrene	9.670	998	0.03
6.	Ethyl-Hexanoate	9.874	999	0.08
7.	*α*-Terpinene	10.323	1017	0.02
8.	1,8-Cineole	10.669	1022	0.03
9.	*D*-Limonene	10.852	1023	0.40
10.	*p*-Cymene	10.957	1025	0.26
11.	*β*-Ocimene (*E*)	11.650	1038	0.07
12.	γ-Terpinene	12.116	1050	0.13
13.	2-Nonanone	13.314	1072	0.11
14.	Terpinolene	13.660	1079	0.10
15.	Linalool	14.144	1086	0.11
16.	Benzyl acetate	16.957	1138	0.05
17.	Benzoic acid, ethyl ester	17.278	1149	0.05
18.	Terpinen-4-ol	17.705	1163	0.06
19.	α-Terpineol	18.495	1175	0.91
20.	Chavicol	21.142	1234	0.06
21.	Geraniol	21.869	1237	0.06
22.	α-Terpinyl acetate	25.298	1333	4.12
23.	Eugenol	26.567	1335	59.87
24.	Geranyl acetate	26.997	1361	0.12
25.	*β*-Caryophyllene	28.563	1415	23.58
26.	Caryophyllene (*Z*)	28.651	1419	0.03
27.	Humulene	29.788	1451	3.74
28.	*γ*-Cadinene	30.370	1507	0.03
29.	Cadina-1(2),4-diene, *cis*	30.500	1526	0.06
30.	Eugenol acetate	30.700	1524	0.04
31.	Germacrene B	31.014	1544	0.10
32.	Caryophyllene oxide	31.313	1573	0.07
33.	*α*-Amorphene	31.469	1688	0.02
34.	*γ*-Muurolene	31.798	1691	0.21
35.	*β*-Selinene	32.015	1715	0.04
36.	α-Selinene	32.540	1725	4.67
37.	*α*-Farnesene	34.699	1745	0.07

Table 2. Clove essential oil composition analyzed by GC-MS of the present study compared with previous studies (content in %age)

S. No.	Compounds	Present study	Hatami et al., 2019	Kennouche et al., 2015	González-Rivera et al., 2016
1.	Eugenol	59.87	87.3	65.36	66.9
2.	*β*-Caryophyllene	23.58	1.36	24.62	24.8
3.	α-Selinene	4.67	-	-	-
4.	α-Terpinyl acetate	4.12	-	-	-
5.	Humulene	3.74	0.19	Trace	3.1
6.	Eugenol acetate	Trace	10.4	5.71	2.7
7.	Caryophyllene oxide	Trace	0.2	-	0.1

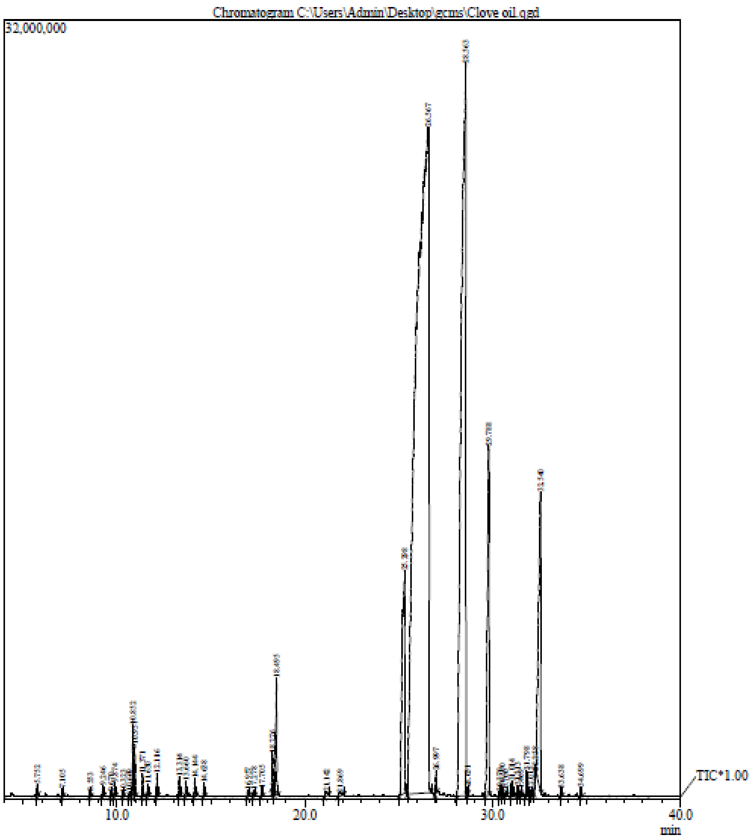

Figure 1. GC-MS spectrum of *S. aromaticum* essential oil

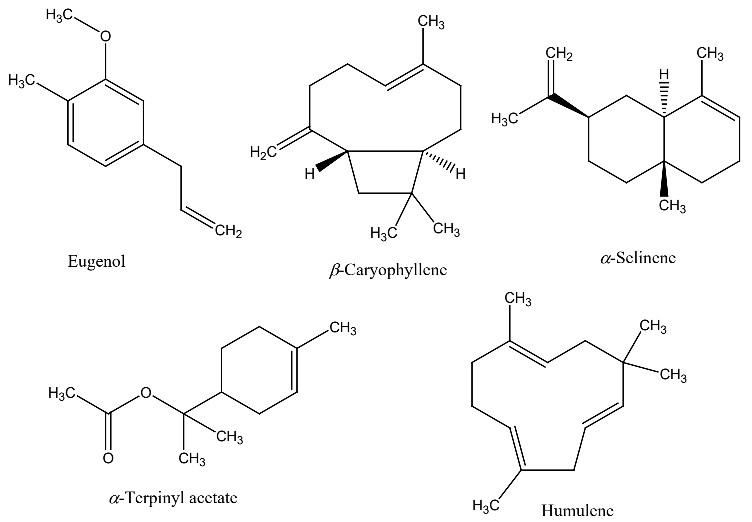

Figure 2. Structures of major chemical compounds present in the essential oil of *S. aromaticum*

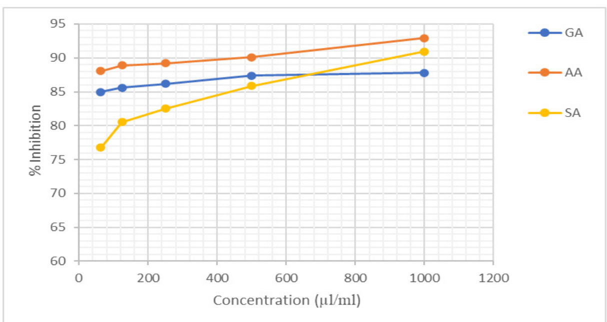

Figure 3. Antioxidant activity of *S. aromaticum* essential oil. where, GA: Gallic acid; AA: Ascorbic acid; SA: *Syzygium aromaticum*

previous reported studies. The difference in the essential oil composition may be due to the extraction condition, geographical location, and environmental conditions (Najibullah et al., 2021; Ahamad et al., 2019).

3.2. Antioxidant activity of Essential oil of S. aromaticum

Various plants have different levels of efficacy in terms of antioxidant activity of Kurdish medicinal plants which are presented in Figure 3. The study revealed that gallic acid, ascorbic acid, and *S. aromaticum* have the most potent antioxidant activity within the inhibition range 84.98 to 87.8%; 88.06 to 92.2%; 76.76 to 90.94%, respectively at a concentration ranging from 62.5 to 1000 µl/ml. *S. aromaticum* has potent antioxidant activity in all concentrations and it was found comparable to standard antioxidant compounds such as gallic acid and ascorbic acid. Spices and condiments are known for their potential antioxidant activity (Martínez-Tomé et al., 2001). The intake of such spices and condiments with food decreases the potential risk of cancer, diabetes, and cardiovascular complications (Kaefer, & Milner, 2008; Li et al., 2023; Ofori-Asenso et al., 2021).

4. Discussion

The study conducted a comprehensive analysis of the essential oil of Clove bud (S. aromaticum) using GC-MS. The hydrodistillation method yielded about 15.67% of colorless essential oil with a distinct aroma. The GC-MS analysis identified 37 chemical compounds, with eugenol (59.87%), β-caryophyllene (23.58%), α-selinene (4.67%), α-terpinyl acetate (4.12%), and humulene (3.74%) as major constituents. Comparative analysis with previous studies showed consistency in major compounds like eugenol and caryophyllene, but the present study revealed unique major constituents, indicating variations due to extraction conditions and environmental factors.

Moving to the antioxidant activity of the essential oil, the study compared the efficacy with gallic acid and ascorbic acid. S. aromaticum demonstrated potent antioxidant activity, ranging from 76.76% to 90.94% inhibition at concentrations from 62.5 to 1000 µl/ml. The results suggested that S. aromaticum's antioxidant activity was comparable to standard compounds, highlighting its potential health benefits. The discussion also referenced the known antioxidant properties of spices and condiments, emphasizing their role in reducing the risk of various health issues. In summary, the study not only provided a detailed analysis of the chemical composition of Clove bud essential oil but also explored its antioxidant potential. The findings contribute to understanding the therapeutic potential of S. aromaticum and emphasize the importance of considering factors such as extraction conditions and geographic variations in studying the chemical composition of essential oils. Additionally, the study supports the notion that incorporating spices and condiments with antioxidant properties, like S. aromaticum, into one's diet may contribute to reducing the risk of certain health complications.

5. Conclusion

Clove bud is extensively used as a spice and condiment worldwide for their characteristic aroma and medicinal benefits. Clove is traditionally used as a carminative and dental analgesic; and it has many bioactivities such as antioxidant, antifungal, anticancer, and anti-inflammatory. The GC-MS analysis of Clove essential oil resulted in the identification of 37 chemical compounds and the essential oil was found rich in eugenol, caryophyllene, α-selinene, α-terpinyl acetate, and humulene. The antioxidant activity of Clove essential oil was found comparable with standard antioxidant compounds such as ascorbic acid and gallic acid. The current study explores the chemical composition of Clove essential oil and its antioxidant potential.

Author Contributions

J.A. conceptualized, performed the experiments and revised the article.

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Competing financial interests

The authors have no conflict of interest.

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