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**Chemical Components and Antioxidant Activity of
Eugenia caryophyllus Essential Oil**

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Abstract

Clove, *Eugenia caryophyllus*; (Myrtaceae) is an aromatic floral bud commonly used as spice. The aim of the study was to extract, identify chemical components and antioxidant of *Eugenia caryophyllus* essential oil. The clove sample was collected from Ibn Msauod market (El-Obeid city, North Kordofan State). The hydrodistillation method has been used to extract the essential oil from the plant sample. Phytochemical screening revealed the presence of alkaloid, terpenoid, flavonoid, tannin, saponin and reducing sugar. Gas chromatography- mass spectrometry (GC/MS) analysis showed sixteen compounds. Eugenol (83.89%), eugenol acetate (13.62%) and trans caryophyllene (1.29%) as the major oil components. Three different concentrations (83, 75 and 50%v/v) of the essential oil and the standard were used to estimate the antioxidant activity by 2, 2 Di (4 -tert -octyl phenyl) - 1 - picryl – hydrazyl (DPPH) free radical. The essential oil and the standard showed a DPPH scavenging activity of (90 and 91 at concentration 83), (84 and 88 at concentration 75) and (0.0013 and 0.077 at concentration 50) respectively.

Keywords: Clove, hydrodistillation, Phytochemical, Ibn Msauod.

Introduction

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (Tap sell *et al* ., 2006) (Lai- pk and Roy , 2004). In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethno medical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant (Fabricant and Farnsworth , 2001). Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, quinine, and opium (Swain and Tony , 1968).

The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization (WHO) estimates that 80% of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available. Clove is aromatic flower buds of a tree in the family Myrtaceae, *Syzygium aromaticum*. It is native to Maluku Islands in Indonesia, and commonly used as spice. Clove is commercially harvested primarily in Indonesia, India, Madagascar, Zanzibar, Pakistan, Sri Lanka and Tanzania. The aroma of the clove is pleasant

yet spicy and can be used to make drawers and closets smell nice. Clove has some medicinal purposes as well as it tastes good in certain dishes like spice cake. Clove like to grow in hot tropical climates like the islands of Indonesia and is an evergreen tree that can reach a height of thirty or forty feet high. Clove is one of the highest sources of manganese. Manganese is vital for metabolism, contributes enzymes, promotes bone strength, and also adds to clove's high Oxygen Radical Absorption Capacity (ORAC), antioxidant value, and also sources for magnesium, calcium, vitamins C and K. Clove is high in fiber also omega-3 is in abundance in clove as well as many phytonutrients that enhance the immune system (Jirovetz and Buchbauer , 2006). Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by fermentation or extraction but the method of steam distillation is most commonly used for commercial production. An estimated 3000 essential oils are known, of which 300 are commercially important in fragrance market. Essential oils are complex mixture comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of these constituents contributes to the beneficial or harmful effects (Vande *et al.*, 1999). The aromatic oil of the clove has a stimulant and irritant effect. Clove oil is an antiseptic in nature and effective against streptococcus and staphylococcus bacteria and therefore it is used in respiratory and digestive diseases. Because of their antiseptic nature they find a place in the preparation of mouth washes, tooth pastes and tooth powder in ear ache and also help fight infections like cold, flu, bronchitis, arthritic pain and athlete's foot (Rockville , 2008). Clove oil is a good remedy for treating on the eye (Inflammation of the eyelids in the form of small growths) (Bullock and S . Harrison , 2002)(Rhayour and Bouchikhi , 2003), in pain from burns and wounds, also help mosquito repellent, and ant killer (Heinrich and Barnes , 2004). Clove oil is an unusually powerful antioxidant. Antioxidant capacity is measure by (ORAC) (Jirovetz and Buchbauer , 2006). Antioxidants are compounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu *et al.* , 1998). Molecules having antioxidant properties are known to be phenolic compounds: acid-phenols or flavonoids and their esters (Rafat and Cillard , 1987). The antioxidative potency of phenolic compound depends on the chemical structure, in particular, electron delocalization on the aromatic nucleus (Kitagawa *et al.*, 1992).

Materials and Methods

Sample Collection:

The clove sample was collected from El-Obeid city market (North Kordofan State-Sudan).

Extraction of Clove Oil:

100 g of clove sample were placed in 2000 ml rounded bottom flask, and 1000 ml of distilled water were added to the flask using a Clevenger type apparatus. The system was heated to 100 C° for about four hours till the volume of the oil above the water layer in the receiver became constant. The oil was pipette, dried over anhydrous sodium sulphate and stored in a dark container in a refrigerator at 4 C° till used (Sukhdev *et al.*, 2008). Yield percentage was calculated as followed: $\text{Volume of oil/Weight of plant sample} \times 100$

Gas Chromatography-Mass Spectrometry (GC-MS) analysis:

GC/MS analysis was carried out with a fisons instrument GC2010, equipped with mass selective detector and quad rupole analyzer. 1to3 μm of the volatile oil was diluted by the addition of 25 ml ethanol. The injection mode is split, and column oven temperature 35.0 C°. The flow control mode by pressure 61.8 Kpa, column flow 1.20ml/min. Helium was used as a carrier gas at a linear velocity39.4cm/sec, the injection temperature was 250C°. The identification of compounds was performed by comparing their mass spectra with data mass spectra library spectra. The identification of compounds was also based on the Kovats retention indices.

Evaluation of Antioxidant Activity of the Extracted Oil:

The effect of extracted oil on 2,2-Di (4 - tert- octylphenyl) - 1- picryl - hydrazyl (DPPH) was assayed using the method of (Shimada *et al.* , 1992) , with some modification. 0.5ml of the test sample was dissolved in 1ml of dimethyl sulfoxide (DMSO) while DPPH, was prepared in ethanol. In 96- well plate, the test sample was allowed to react with DPPH free radical for half an hour at 37°C. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrometer. Percentage radical scavenging activity by sample was determined in comparison with a DMSO treated as control group and propyl gallate(PG) as a standard. All test and analysis were run in triplicate.

Phytochemical Screening:

Phytochemical examinations were carried out for the extracted oil as per the standard methods. Alkaloids and terpenoids were determined according to Siddiqui and Ali (1997). Flavonoids, tannins, reducing sugar and saponins were determined according to Iyengar (1995).

Results

Extracted Oil:

The percentage of the extracted oil was shown in table (1).

Table (1): Yield % of the extracted oil

Sample	Weight of Sample	Volume of Oil	Yield%
Clove	100g	9.5ml	9.5%

Gas Chromatography-Mass Spectrometry (GC-MS) analysis:

Table (2) Shows the compounds identified in the extracted oil. 16 different compounds were identified.

Table (2): Identified compounds by GC/MS in the extracted oil

Peak No.	R.time	Area%	Molecular Weight	Chemical formula	Compound name
1	8.747	0.05	106	C ₈ H ₁₀	Benzene,1,3- dimethyl
2	9.525	0.04	106	C ₈ H ₁₀	Benzene,1,2- dimethyl
3	14.154	0.19	154	C ₁₀ H ₁₈ O	1,8-Cineole
4	14.521	0.03	172	C ₁₀ H ₂₀ O ₂	Octylacetate
5	16.137	0.02	142	C ₉ H ₁₈ O	Methylheptyl ketone
6	18.855	0.05	154	C ₁₀ H ₁₈ O	4-Terpineol
7	19.377	0.07	152	C ₈ H ₈ O ₃	Benzoic acid 2-hydroxy methyl ester
8	21.415	0.06	134	C ₉ H ₁₀ O	4-Allylphenol
9	23.651	0.04	196	C ₁₂ H ₂₀ O ₂	alpha-Terpinyl acetate
10	24.147	83.89	164	C ₁₀ H ₁₂ O ₂	Eugenol
11	24.334	0.14	115	C ₅ H ₉ NS	4-(Methyl Sulfanyl) butane nitrile
2	25.608	1.29	204	C ₁₅ H ₂₄	Trans- Caryophyllene
13	26.482	0.17	204	C ₁₅ H ₂₄	alpha- Humulene
14	28.259	13.62	206	C ₁₂ H ₁₄ O ₃	Eugenol acetate
15	29.699	0.31	220	C ₁₅ H ₂₄ O	Caryophyllene oxide
16	30.319	0.03	220	C ₁₅ H ₂₄ O	Humulene oxide

Where: R.Time=Retention time, Area=Concentration

DPPH radical Scavenging Assay (Antioxidant):

The percentage of DPPH radical scavenging activity of essential oil was shown in table (3).

Table (3): Effect of Essential Oil on DPPH assay:

Sample	% of activity \pm SD	% Concentration of sample and standard
EO	90 \pm 0.01	83
PG	91 \pm 0.03	
EO	84 \pm 0.02	75
PG	88 \pm 0.01	
EO	0.0013 \pm 0.04	50
PG	0.077 \pm 0.01	

EO: essential oil, PG: propyl gallate (standard)

Phytochemical Screening:

Phytochemical screening of the extracted oil revealed the presence of tannins, saponins, terpenoids,

flavonoids, reducing sugar and alkaloids as shown in table (4).

Table (4): Phytochemical constituents of the extracted oil

Metabolite	Concentration
Tannins	+++
Saponins	+++
Terpenoids	+++
Flavonoids	++
Reducing sugar	++
Alkaloids	+

+++ = High concentration

++ = Moderate concentration

+ = Low concentration

Discussion

The extraction of 9.5% essential oil from 100g of clove this mean high content of essential in the clove compared to extracted oil from other plants reported in the literature. Phenolic compounds and eugenol are reported to possess antioxidant and free radical scavenging activities. The percentage of DPPH radical scavenging activity presented in table (3). The essential oil exhibited a maximum DPPH scavenging activity of 90% at 83% whereas for the standard (propyl galate) was found to be 91% at 83%. The DPPH radical scavenging activity of the essential oil increases with increasing concentration. The presence of saponins in this plant may account for management of excess cholesterol and thus reduce the risk of cardiovascular disease. Flavonoids have been shown to exert potent antioxidant activity against the superoxide radical. The tannins and alkaloids have been shown to exert antibacterial activities. The presence of these metabolites probably explains the various uses of this plant in traditional medicine.

Conclusion

From the results obtained in this study, it is concluded that the essential oil which contain high percentage of eugenol, exhibits high antioxidant and free radical scavenging activities. This indicates that this oil is a significant source of natural antioxidant and support the traditional use of clove and development of commercial drugs.

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