

## Chemoprofiling of clove fruit oil and its antioxidant potential

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**Abstract:** Clove (*Syzygium aromaticum* L.) Merrill & Perry syn. *Eugenia caryophyllata* (L.) is an important spice with versatile applications. The dried unopened flower buds of clove are used as the spice. Its flower, leaf and stem yield essential oil. Clove fruits are one of the biological adulterants found in clove buds. In the present study the chemical profile and antioxidant potential of clove fruits was evaluated and compared with that of flower buds. Hydrodistillation of clove fruits yielded 1.1 - 1.9% essential oil and flower buds, 8% oil. The major component of the oil was eugenol. The antioxidant potential of fruits and buds was compared by DPPH assay, phosphomolybdate assay and ferric ion reducing power method.

### Introduction

Clove *Syzygium aromaticum* (L.) Merrill & Perry (synonym: *Eugenia caryophyllata* L.) commonly known as clove, is a medium size tree of the Myrtaceae family. Its unopened flower buds are the spice of commerce. Clove is one of the most valuable spices that has been credited with diverse applications. It is used for preservation of food, in flavoring, perfumery, medicinal preparations and aromatherapy and as anesthetic and analgesic (Anderson *et al.* 1997, Robenorst 1996, Matan *et al.* 2006, Kildeaa *et al.* 2004,). The biological activity of clove has been investigated on several microorganisms and parasites, including pathogenic bacteria, Herpes simplex and hepatitis C viruses. Clove essential oil possesses antimicrobial, antioxidant, antifungal, antiviral, antiinflammatory, cytotoxic, insect repellent and anesthetic properties (Chaieb *et al.* 2007, Pinto *et al.* 2009, Leela and Sapna 2008). It is widely used in agricultural applications to protect foods from micro-organisms during storage, which might have an effect on human health, and as a pesticide and fumigant. As a functional ingredient, it is included in many dental preparations and it has also been shown to enhance skin permeation of various drugs.

The essential oil extracted from the dried flower buds of clove, is used to relieve pain and promote healing and also finds use in the fragrance and flavouring industries. The composition of leaf, bud and stem oil has been investigated. The main constituent of the essential oil is eugenol which is credited with a variety of uses (Leela & Sapna 2008). Recent finding is that eugenol ameliorates diabetic nephropathy in streptozotocin induced diabetic rats (Garud & Kulkarni 2017). The antioxidant activity of clove leaf essential oil and was studied by Jirovetz *et al.* (2006). To the best of our knowledge there is no detailed study on the constituents of oil of fruits. The composition of fruit oil and its antioxidant activity are reported here.

### Materials & methods

Extraction of essential oil: Clove fruits and dried flower buds were collected from Thottilpalam, near Kozhikode. The essential oil was extracted by hydrodistillation for 3-4 hrs and the oil was collected, dried over anhydrous sodium sulphate and stored in refrigerator until further analysis was carried out.

Preparation of solvent extracts: Dried clove fruits (310 g) were powdered and extracted with hexane followed by methanol for 30h each and the extracts were evaporated to dryness and the percentage yield was recorded.

Antioxidant activity: The essential oil and extracts were dissolved in methanol and the antioxidant activity was determined by. DPPH method (Braca *et al.* 2001), phosphomolybdate method (Prieto *et al.* 1999) and ferric ion reducing power method.

DPPH radical scavenging assay : The method suggested by Braca *et al.* 2001 was used for the determination of antioxidant activity. Different aliquots (1-30 µg/ml) of sample were taken in test tubes and diluted to 4ml with methanol. Then 1ml of 0.004% DPPH reagent was added to each test tube and mixed well. The tubes were then kept in dark for 30 minutes and the absorbance of the solution was measured using Shimadzu UV- visible spectrophotometer, UV-1800 at 517nm. Methanol served as the control. BHA was used as standard antioxidant. DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging capacity (\%)} = \frac{(\text{Absorbance of control}) - (\text{Absorbance of sample})}{\text{Absorbance of control}} * 100$$

### **Ferric ion reducing power method**

Varying concentration of the extracts (10-30µg/ml) was pipetted out in to test tubes and diluted to 1 ml with distilled water. The samples were mixed with 2.5ml phosphate buffer (0.2M, pH 6.6) and 1% freshly prepared aq.potassium hexacyanoferrate (2.5ml) and incubated at 50°C for 30 min. The reaction was terminated by adding trichloroacetic acid (10M, 2.5ml) and the mixture was centrifuged at 3000 rpm for 20 mts. The supernatant (2.5ml) was mixed with equal volume of distilled water and 0.5ml of 0.1% freshly prepared aq. FeCl<sub>3</sub> solution (0.5ml, 0.1%). The absorbance of the mixture was measured at 700 nm against reagent blank. Ascorbic acid (0.25-1.0mM) was used as standard. The antioxidant potential was expressed in terms of MAAE (Molar Ascorbic Acid Equivalence). BHA was used as positive control.

### **Phosphomolybdate assay**

The total antioxidant capacity of the extracts was estimated based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto *et al.*, 1999). Varying concentration (10-30 µg/ml) of the extracts was pipetted out in to test tubes and diluted to 3ml with methanol. 1 ml phosphomolybdate reagent (0.6M sulphuric acid, 28 mM disodium hydrogen phosphate and 4 mM ammonium molybdate) was added and

incubated at 95°C for 90 min. After incubation the absorbance of the solution was read at 695 nm. Ascorbic acid (0.2-1mM) was used as the standard for preparation of standard curve. The antioxidant potential was expressed in terms of MAAE (Molar Ascorbic Acid Equivalence). BHA was used as standard antioxidant.

### **Gas chromatography-mass spectroscopy analysis**

Essential oil was analyzed using a gas chromatograph (Shimadzu GC 2010) equipped with mass spectrometer (Shimadzu MS QP-2010) and RTx-wax column, (30m×0.25mmid×0.25µm). Injection port temperature: 250°C, Detector Temp. 220 °C, Interface Temp: 250 °C

Carrier gas was helium with linear velocity of 48.1 cm/s at a flow rate of 1 ml/min with split ratio: 50 , ionization energy: 70 eV and mass range: 40-650 amu. The column temperature was programmed as follows: At 60°C - 5min; 60-110°C @ 5 °C /min;110-200°C @3 °C /min;200-220°C @5 °C /min; at 220°C 5min.

### **HPLC analysis of clove fruit extracts**

The HPLC analysis of fruit extract (5mg/ml) was carried out using High Performance Liquid Chromatography (Shimadzu) LC-10ATVP equipped with SPD-10AVP, SCL-10AVP, and RP C-18 column (5µ). Methanol: acetonitrile: water (10:50:40) was used as the mobile phase with a flow rate of 1 ml/min, at 280 nm. 20 µl sample was injected. Authentic standard of eugenol (Sigma) was also injected under the same conditions.

## **Results and discussion**

Clove fruits and buds contained 1.1-1.9% essential oil and 8% oil respectively (Table 1). Dried clove fruits yielded 14.5% and 16.7% hexane and methanol extracts respectively. Table 2 shows the composition of the essential oil. Ten constituents representing more than 97% of the essential oil of clove fruits were characterized. The major components were eugenol, 1-(6-hydroxy 2,4, dimethoxy-5-methylphenyl) ethanone and t-caryophyllene. However quantitative differences were observed with respect to fruits at different maturity stages. Freshly harvested purple coloured fruits had higher eugenol (79.89 %) compared to the dried ones (67.24 %). Dried immature fruits had lowest eugenol content (62.18%). 1-(6-hydroxy 2,4, dimethoxy-5-methylphenyl) ethanone was found in higher level (31.7%) in immature dried clove fruits which was followed by mature dried ones (23.8 %) and freshly harvested ripened fruits (11.84 %). The essential oil from dried clove buds was dominated by 76.24 % eugenol, 15.65 % eugenyl acetate and 5.43% t-caryophyllene besides  $\alpha$ - humulene and caryophellene epoxide. The fruit oil contained 1-(6-hydroxy 2,4, dimethoxy-5-methylphenyl) ethanone which was not detected in bud

oil. Hence 1-(6-hydroxy 2,4, dimethoxy-5-methylphenyl) ethanone could be possibly used as marker compound for detection of adulteration of bud oil.

Antioxidant potential of essential oils and solvent extracts of clove fruits was determined by three methods and are indicated in the Table 3. By DPPH assay the capacity of the extracts to scavenge the DPPH free radicals is measured and is expressed as IC<sub>50</sub> value which indicated the minimum concentration of the extract required to remove 50% of free radicals. Hence lower IC<sub>50</sub> value shows higher efficacy of the extract. The IC<sub>50</sub> values of the extracts varied between 13.9-32.0 µg/ml. Essential oil showed higher activity compared to solvent extracts and antioxidant activity of clove bud oil was higher than that of fruit oil (IC<sub>50</sub> = 14.5 µg/ml). The chief component of the oil eugenol (IC<sub>50</sub> = 5.6 µg/ml) and BHA (IC<sub>50</sub> = 5.4 µg/ml) had comparable antioxidant activity.

Antioxidants act as reducing agent by donating electrons and thereby preventing oxidative damages. In ferric ion reducing power method antioxidant substances of extracts reduce the Fe<sup>3+</sup> ion/ferricyanide complex to Fe<sup>2+</sup> form, which is measured by absorbance at 700nm. By FRAP method the antioxidant potential of the extracts varied from 0.25-4.60 MAAE/ g extract (Molar Ascorbic acid equivalents/g extract). Among the extracts clove fruit oil exhibited highest antioxidant potential (4.08-4.60 MAAE/ g extract) than clove bud oil (3.75 MAAE/ g extract), BHA and eugenol. Higher antioxidant activity of clove bud oil compared to the synthetic antioxidants was reported by Gulcin *et al.* (2012) also.

Total antioxidant activity of extracts determined by phosphomolybdate assay varied from 0.13–7.15 MAAE/g extract (ascorbic acid equivalents/g extract) with bud oil showing the highest antioxidant activity. Antioxidant activity of clove essential oils and eugenol (5.15-7.33 MAAE/g extract) was higher than the synthetic antioxidant BHA (3.68 MAAE/g extract). This is in accordance with the observation of Gulcin *et al.* (2012) on clove bud oil. Hexane extract exhibited higher antioxidant capacity by all methods compared to methanol extract of clove fruits. HPLC analysis of the hexane extract of fruits showed eugenol as the predominant compound although eugenol was present in methanol extract also. Our studies indicated that the antioxidant property of the fruit oil and hexane extract of fruits was mostly contributed by the component eugenol. Dorman *et al.* (2000) also reported that clove and eugenol possessed antioxidant activity comparable to that of the synthetic antioxidants, BHA and pyrogallol.

## Conclusion

In the present study the antioxidant potential and chemical profile of clove fruits and buds were compared. The essential oil content of clove fruits was low compared to that of buds. Eugenol was the predominant component of essential oil of clove fruits, clove buds and hexane extract. The major components of fruit oil were eugenol and 1-(6-hydroxy 2,4-dimethoxy-5-methylphenyl)ethanone and t-caryophellene where as in buds eugenol, eugenyl acetate and t-caryophellene dominated. Eugenol, has been widely accepted as a safe molecule and used for its anesthetic and analgesic action in dentistry. It also possesses significant anti-inflammatory and cardiovascular properties and is a promising candidate for industrial applications, and design of new

drugs (Pramod *et al.* 2010). The study indicated that clove fruit is another source of eugenol which is considered as a safe food additive (Kamatou *et al.* 2012).

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